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CRITICAL REVIEW: THE MECHANISM OF HAEMOSTASIS<sup>1</sup>

By R. G. MACFARLANE

(From the Pathological Department, British Post-Graduate Medical School)

With Plates 1 to 3

*Introduction*

Of the many ills that befall mankind, uncontrollable bleeding is probably the most alarming. It is fortunate, therefore, that the normal human body is equipped with a mechanism of defence against haemorrhage so efficient and unobtrusive that its action is apt to be taken for granted. It is not thought remarkable that the minor injuries of everyday life are normally accompanied by only the most transitory bleeding. The surgeon, making his incision, needs only to secure the larger vessels, and he relies, usually unthinkingly, upon nature to seal the countless capillaries, venules, and arterioles that he has also divided. It is only when the haemostatic mechanism fails that the supreme importance of its normal working is fully appreciated. Such a failure is responsible for the 'haemorrhagic diatheses', and in some of these conditions the inability of every therapeutic effort to check a slow ooze of blood that would be rapidly and spontaneously controlled in a normal person provides an impressive demonstration of the normal haemostatic efficiency. In fact, it is precisely the type of bleeding so readily arrested in the normal subject that is so persistent in the haemorrhagic states, and an injury that would usually be considered trivial is frequently the cause of dangerous or even fatal haemorrhage. It is clear that in these states there is a breakdown of a mechanism mainly concerned with the arrest of bleeding from the minute vessels, the constant operation of which makes normal life possible.

In beginning a study of this mechanism, it is remarkable to observe that the generally accepted view of the actual way in which bleeding is arrested appears to rely almost entirely on observations made nearly sixty years ago. In 1882 Hayem made a small incision in the jugular vein of a dog; when bleeding had stopped spontaneously, he observed that the opening was plugged by a mass, which he removed and examined. He found that it was composed of a mixture of fibrin and platelets, the formation of which had resulted, he supposed, in the cessation of the bleeding. While there is no reason to doubt the importance of the observation, or the validity of the deduction made from it, it is perhaps unfortunate that a sweeping generalization of Hayem's view regarding this particular experiment should have been applied to the haemostatic process as a whole, and accepted almost without criticism. It is now commonly supposed that the spontaneous arrest of any bleeding is brought

<sup>1</sup> Received May 25, 1940.

about by the formation of a blood-clot or of a mass of agglutinated platelets. Such a conception seems reasonable enough at first sight, since in conditions in which coagulation of the blood is defective, or in which the platelets are reduced in numbers, there is usually a tendency to bleed persistently. Yet many of the findings in these haemorrhagic states cannot be reconciled with such a hypothesis, as will be shown by an attempt to provide a satisfactory answer to the following questions :

1. If the platelets can stop bleeding by agglutination into masses, why does haemorrhage occur in haemophilia, in which they are present in normal numbers ?

2. If the coagulation of the blood can stop bleeding, why does bleeding persist in purpura haemorrhagica, in which the blood outside the body clots firmly in the normal time ?

3. If it is argued that both normal platelet numbers and normal blood-clotting are needed to produce haemostasis, why does severe or even fatal bleeding occur in telangiectasia, in athrombocytopenic purpura, and in some forms of haemorrhagic jaundice, when in such cases the platelets are normal in number and there is no delay in the clotting-time of the blood ?

4. What are the factors involved in the investigation known as the bleeding-time ? Why is the bleeding-time normal in haemophilia and prolonged in the purpuras, whether the platelets are reduced or present in normal numbers ?

5. Why does splenectomy often result in a remission of the haemorrhagic symptoms in cases of thrombocytopenic purpura, though the platelet count may remain at, or return to, its original low level ?

6. If both purpura haemorrhagica and haemophilia are due to a generalized failure of the haemostatic system, why are the haemorrhages in the two conditions so different in their character and distribution ?

In view of the difficulties raised by these questions, it would seem that some revision of the current ideas on the mechanism of haemostasis might profitably be attempted. The present unsatisfactory position is not due to any lack of data, for a considerable mass of relevant evidence is available for review. This is provided partly by the laboratory, and partly by the clinical findings in the haemorrhagic states which, in fact, constitute a naturally controlled experiment. In this communication such evidence and the results of some personal observations will be examined, and it is hoped to put forward a hypothesis that will conform more closely to the observed facts and explain some of the anomalies that exist in this particular field.

### *The Conditions for Haemostasis*

Before beginning a detailed examination of the factors concerned in the control of bleeding from the minute vessels it is necessary to consider just what the production and maintenance of haemostasis entails. A superficial abrasion of the skin may be taken as an example, being the type of injury which normally stops bleeding in a few seconds, but which may bleed for days or even weeks in certain of the haemorrhagic diatheses. The vascular

supply of the skin is derived from the subpapillary arterial plexus, from which numerous arterioles arise to supply the skin capillaries. An arteriole may supply one or more capillaries, each of which after running towards the surface doubles back in the form of a loop and empties itself into a subpapillary plexus of venules, which in turn communicates with a series of venous anastomoses at increasingly deep levels (Spalteholz, 1893). Valves are not found in the veins of the true skin, and as there is no sharp differentiation between the arterioles, capillaries, and venules in this situation, Lewis (1927 *a*) has called them all 'minute vessels'. The number of capillary loops in a given area of skin varies according to the site from about 16 to 64 per square millimetre (Wetzel and Zotterman, 1926), so that even a small injury involves a large number.

The pressure and conditions of flow in these vessels must be considered. Landis (1930), by means of micro-pipettes, estimated the pressures in various parts of the capillaries. He stated that at the arteriolar end the average pressure was 32 mm. of mercury, and at the venular end 12 mm. There is thus a fall in pressure due to friction of 20 mm. from one end of the capillary to the other. In the arterioles an even greater reduction in pressure occurs, probably to the extent of about 60 mm. of mercury. It is important to realize that since these reductions in pressure are dynamic and are entirely dependent on the flow of blood, any obstruction that reduces the flow will raise the pressure on the arterial side in the vessels concerned. In the case of one of several capillaries supplied by a single arteriole, an obstruction to its outflow will raise the pressure within it to that of its arteriolar supply, that is, to about 30 mm. of mercury. When, however, an arteriole supplies a single capillary, the change in pressure that follows complete obstruction of flow through the latter vessel is much greater, since throughout the length of both capillary and arteriole there will be cessation of flow and a pressure equalling that of the arterial plexus that supplies them. Similar conditions will follow in the case of several capillaries rising from one arteriole if all the former are blocked.

In a superficial abrasion it may reasonably be assumed that the bleeding occurs from capillary loops that have been severed. Since there are no valves in the superficial venous anastomoses, blood will flow from the venular as well as the arteriolar limbs of the damaged capillaries. The haemostatic mechanism has to arrest such a flow and, at the instant of arrest, withstand the ordinary capillary pressure, but immediately after the flow is stopped, static pressures are instituted, and it is probable that many of the affected capillaries will be subjected to a pressure equal to that of the subpapillary arterial plexus, a fact that must be remembered when the mechanism by which haemostasis is produced is considered.

#### *The Haemostatic Factors*

Stoppage of the flow of blood can be brought about by the formation of a solid plug in the wound, reduction of the pressure of the issuing blood, or

by the contraction of the damaged vessels. The formation of a solid mass in the wound, or in the mouth of the bleeding vessels, may be brought about by the agglutination of the platelets and the coagulation of the blood. Reduction in the pressure of the issuing blood may follow a general fall in the blood-pressure, an event that, since it usually occurs only if a large amount of blood has been lost, can hardly be regarded as a part of the normal mechanism controlling capillary bleeding. Pressure may, however, be reduced locally by the contraction of arteries or arterioles supplying the wounded capillaries, a possibility that will be considered later. The contraction of the severed capillaries themselves is another possibility, and one to which little attention has been paid, although it may play a part of fundamental importance in the normal mechanism. These factors will now be examined in greater detail.

*The coagulation of the blood.* Blood coagulation has been the subject of an intensive and sometimes bitter controversy that has persisted for the greater part of a century, and even now schools of thought exist that can find practically no basis of agreement. As far as the haemostatic system is concerned the important point, and one that is so far undisputed, is that coagulation of normal shed blood does in fact occur. What is the evidence that clotting of the blood issuing from a wound is concerned with the arrest of bleeding? The fact that a wound which has ceased to bleed is often filled by a mass of clotted blood naturally suggests the reason for the haemostasis. Moreover, those conditions in which a serious defect of the clotting mechanism exists are invariably associated with a liability to persistent bleeding, and in some of these it is impossible to demonstrate any abnormality apart from the clotting defect.

Experimentally also, it has been found that defective clotting in animals is accompanied by bleeding. Duke (1911) rendered the blood of dogs incoagulable by defibrination. He observed that these animals tended to bleed uncontrollably from small cuts and operation wounds. Hawkins and Whipple (1935) found that dogs with experimental bile-fistulae developed a coagulation defect and a tendency to bleed. In rabbits it can be shown that prolongation of the coagulation-time is followed by persistent bleeding from certain wounds. Using the anticoagulant dye 'chlorazol fast pink' described by Huggett in 1934, I observed the effect of inhibition of clotting on the haemorrhage from large wounds and small punctures. In the first case the animal was anaesthetized, injected intravenously with the dye, and samples of blood were taken at intervals during the experiment to determine the anticoagulant effect. An incision about an inch long was made in the flank, extending into muscle. It was found that if the large divided vessels were secured, very little blood was lost, and after a few minutes the flow ceased. Haemostasis was not maintained, and after about half an hour bleeding restarted and persisted for several hours. The blood coagulation time throughout the experiment was over 24 hours. Unless the dye had some other effect in addition to its anticoagulant action, it appears that, under

these conditions, clotting is necessary for the maintenance of haemostasis. In the case of the small punctures, however, the coagulation of the blood does not seem to be important. One hundred estimations of the time required for the arrest of bleeding from punctures made with a small stylet were carried out in normal rabbits. The wounds were 3 mm. long and just perforated the skin. The issuing blood was removed every 15 sec. with the edge of a filter paper, so that a graphic record of the experiment was obtained. The mean normal bleeding-time was found to be 1.06 min. ( $\sigma = 0.129$ ). Two rabbits were then given anticoagulant, heparin in one case, and 'fast-pink' in the other, enough of each being used to render the blood incoagulable, and estimations of the bleeding-time made. The mean of 10 values in the rabbit receiving heparin was 0.875 min. In the animal receiving the dye the mean of 10 values was 1.3 min., and these included one reading of 3.75 min. obtained when a small vein had been punctured. The final haemostasis was permanent, and the small wounds dried up in an hour or less.

These observations can be correlated with clinical experience, for in some of the severest haemorrhagic states associated with defective coagulation bleeding from small punctures ceases as quickly as in the normal subject. In other conditions the bleeding-time is greatly prolonged, so that the patient may bleed for hours from a needle puncture, despite the fact that no coagulation defect can be demonstrated. The inference is, therefore, that not only is coagulation of the blood unnecessary for the control of bleeding from wounds of this nature, but that its apparently normal operation is incapable of arresting even the haemorrhage from a needle puncture. The latter conclusion is not altogether surprising when the haemostatic efficiency of coagulation is considered more closely. The actual time required for clotting is important, but unfortunately there are many different methods of measurement, and consequently a wide variety of 'normal' figures. The average normal coagulation time as measured by the method of Dale and Laidlaw (1911) is about  $1\frac{1}{2}$  min., while by that of Lee and White (1913) it is as long as 8 min. These figures refer to the clotting of the blood under the conditions of the test used, and their relation to the coagulation of the blood under natural conditions cannot be specified. It would seem that the methods that use capillary blood, as for instance those of Wright (1893), Dale and Laidlaw (1911), and Gibbs (1924), give a closer approximation to the 'natural' time than the methods employing venous blood in which admixture with tissue factor is avoided. From experiments in which an excess of tissue factor is added to blood (Quick, Stanley-Brown, and Bancroft, 1935) it appears unlikely that normal blood under the best natural conditions can clot in less than 20 or 30 sec., and it is probable that the usual time is between this figure and the  $1\frac{1}{2}$  min. obtained with capillary blood *in vitro*.

It is difficult, therefore, to see how clotting alone can arrest a flow of blood in the time normally required by the process of haemostasis. In the case of a severed capillary opening on to the surface of a wound, the first

stimulus to clot that the blood receives is contact with the damaged endothelium at the cut margin. Since clotting can take place only after a period of  $\frac{1}{2}$  to 1 min. has elapsed from the moment of this contact, coagulation of the main mass of flowing blood will occur, not at the mouth of the vessel where it might be effective, but at the point to which the rate of flow has carried it in this interval. Fibrin can form at the bleeding-point only by coagulation of the molecular film of plasma remaining stationary in contact with the damaged tissue. The coagulation of successive layers of plasma, together with the deposition of platelets, may well, if the flow of blood continues for sufficient time, result in the building up of a mass such as that described by Hayem, and which is analogous to the white thrombus that slowly forms intravascularly on areas of damaged endothelium. Under the conditions of Hayem's experiment such a mass would result in haemostasis, because the pressure in the jugular vein is extremely low and bleeding probably continued for a considerable time, but it seems most unlikely that such a process could close the mouths of bleeding capillaries in the minute or so that is required for normal haemostasis to occur. It is also unlikely that the red clot so often seen filling a wound has actually arrested the bleeding, for the delicate strands of newly formed fibrin could hardly withstand the pressure to which they would be immediately subjected. During bleeding, a wound is continually refilled with fresh blood that has no time to clot *in situ*. The red clot in this situation would seem to be analogous to the red thrombus, usually formed under conditions of stasis, and it is more likely to be the result and not the cause of haemostasis.

From these considerations it appears that coagulation of the blood, though incapable by itself of arresting bleeding, has an indispensable part in the maintenance of haemostasis produced by some other factor, except in the case of punctured wounds. If this be so, the rate of clotting is less important to the haemostatic efficiency than is usually supposed, and other attributes of the clot, such as toughness and retractibility, may have a greater influence. Clot retraction, occurring as it does  $\frac{1}{2}$  to 1 hr. after coagulation, could not be concerned in the initiation of haemostasis, but may well have a part in its maintenance. The newly formed blood-clot is normally a soft, friable mass, but after retraction, during which it shrinks to about half its original volume, it becomes relatively tough and elastic. A deficiency in the retractibility of the clot is often, but not always, associated with a tendency to bleed (Macfarlane, 1939). It is unfortunate that, at present, there is no satisfactory method for measuring the toughness of blood-clots.

*The blood platelets.* In 1872 Zahn noticed that an area of injured endothelium in a vessel through which blood was flowing became covered by a hyaline deposit. Bizzozero, in 1882, made a similar observation, and identified the deposit as a mass of agglutinated platelets, these findings being confirmed by Eberth and Schimmelbusch in 1885. About the same time Hayem (1882) carried out the experiment already described, and was

so impressed by the haemostatic possibilities of the platelets that he suggested that a shortage of platelets would be accompanied by haemorrhagic symptoms. This prediction was soon fulfilled by the observations of Kraus (1883), Denys (1887), and by Hayem himself in 1895, during the investigation of cases of purpura haemorrhagica. In 1910 Duke devised the test known as the 'bleeding-time', a measurement of the efficiency of the haemostatic mechanism in actual operation. He noted that when there was shortage of platelets there was usually a prolongation of the bleeding-time, and suggested that the bleeding-time was actually controlled by the number of available platelets which adhered to the openings in wounded capillaries (Duke, 1912). In 1907 Cole prepared a specific anti-platelet serum, and in 1915 Ledingham and Bedson injected a similar serum, derived from a rabbit immunized against guinea-pig platelets, into guinea-pigs, an experiment that was repeated by Lee and Robertson (1916). This serum produced a marked reduction in the number of circulating platelets and haemorrhagic purpura, suggesting a causal relationship between thrombocytopenia and haemorrhage. Duke (1912) obtained similar results with diphtheria toxin and benzol. Though the case for the supposition that the platelet is an essential component of the haemostatic system therefore seems to be a strong one, it is necessary to examine the evidence more closely.

For similar reasons to those discussed in connexion with the clotting of the blood, it seems unlikely that the platelets can achieve the result attributed to them in the time required for normal haemostasis. They undoubtedly do adhere to foreign surfaces, and to each other, forming masses that might in time produce haemostasis. In the case of a severed capillary, however, the only foreign surface to which the platelets can effectively adhere is the rim of endothelium at the cut end of the vessel, and it appears unlikely that the few platelets brought into actual contact with this small surface could form into a mass which unaided by any other mechanism could occlude the opening within the minute or so required by the normal haemostatic process, or could withstand the pressure resulting from blocking of the vessel. Apart from these theoretical criticisms, there are others of greater weight. Duke's contention that the platelets were directly concerned in haemostasis was largely based on the supposed correlation between the bleeding-time and the platelet-count. Though this correlation is often close, there are divergencies so wide that there must be grave doubts as to the existence of the causal relationship he inferred. For instance, Buckman and Hallisey (1921) described a case of pernicious anaemia in which the platelets were reduced to 32,000 per c.mm. (the normal by their method being 250,000 to 300,000 per cm.) though the bleeding-time was  $3\frac{1}{4}$  min., and Dyke and Stewart (1931) record three cases in which the platelet-counts were 17,000, 22,000, and 29,000 per c.mm., without any haemorrhagic manifestations. Sanford, Leslie, and Crane (1936) observed a thrombocytopenia of 10,000 per c.mm. in a woman shortly after delivery, and of 20,000 per c.mm. in her child, though in both cases there was no prolongation of the bleeding-time. Rosenthal



(1928) quotes the following series of simultaneous estimations of the bleeding-times and platelet-counts in cases of leukaemia and chronic thrombocytopenia.

Platelet-count, per c.mm.	Bleeding-time
5,000	1 min.
5,000	2 "
6,000	2 "
4,000	3 "
10,000	1 "
2,000	6 "
36,000	3 "
13,000	3 "
25,000	1 "

The platelets, therefore, may be reduced to very low levels without an impairment of the haemostatic efficiency. Conversely, the bleeding-time may be greatly prolonged though the platelets are present in normal numbers. The following instances, examples of athrombocytopenic purpura, are reported :—

Platelet-count, per c.mm.	Bleeding-time	Authors
200,000	30 min.	Bailey and McAlpine, 1935
315,000	10 "	Bain, 1939
680,000	30 "	Buckman, 1928
500,000	30 "	Buckman, 1928
545,000	25 "	Fowler, 1937
240,000	28 "	Kennedy, 1928
300,000	60 "	Kennedy, 1928
350,000	15 "	Kugelmass, 1932
280,000	120 "	Little and Ayres, 1928
290,000	24 hrs.	Mathewson and Cameron, 1937
204,000	10 min.	Rosenfeld, 1921
240,000	10 "	Rosenthal, 1928
350,000	35 "	Rothman and Nixon, 1929
342,000	11 "	Schlicke and Hall, 1938

These show that persistent bleeding from small wounds is often associated with an abundance of platelets.

The results obtained with anti-platelet sera and other purpurigenic agents are also less favourable to the accepted view than appears at first sight, although they are often quoted in its support. The haemorrhagic symptoms that follow the administration of these agents are usually represented as being directly due to the reduction in the number of circulating platelets. Such an inference is only justified, firstly, if it can be shown that experimental thrombocytopenia is always followed by these symptoms, and, secondly, that the agent has no other action that might result in bleeding.

With regard to the first condition, it has been found by Roskam (1922) and by Bedson (1922) that thrombocytopenia can be induced without a tendency to bleed. Roskam, injecting gelatine solution intravenously into dogs, reduced the platelets from 408,000 to 3,100 per c.mm. without a marked increase in the bleeding-time. Bedson (1922) also reduced the platelets by an injection of 'agar-serum' without producing haemorrhagic tendencies. Bedson and Johnston (1925) found that anti-bone-marrow serum produced a thrombocytopenia of 40,000 platelets per c.mm., but no purpura or other haemor-

rhagic symptoms. Finally, Bedson (1924) has made the extremely interesting observation that anti-platelet serum failed to produce purpura in guinea-pigs that had been recently splenectomized, even though the platelets were reduced to 22,000 per c.mm. The second condition is also not fulfilled. Tocantins (1936 *a*) found that anti-platelet serum prepared in the same way as that used by the other workers produced a spreading area of haemorrhage when injected into the skin of dogs, and in fact he used this reaction for the titration of this serum. This local production of haemorrhage can be due only to a direct action on the tissues, and it is impossible to exclude a similar, generalized action when larger amounts are used. The same objection obviously applies to other purpurigenic agents, such as diphtheria toxin, streptococcal toxins, and benzol.

The platelets are, of course, concerned in the inception of blood coagulation, probably on account of their kinase content. They are apparently present in considerable excess for this purpose, however, since Mackay (1931) could find no correlation between the clotting-time and the platelet counts in a variety of conditions in which the latter varied from 2,000 to 1,000,000 per c.mm. The platelets are also concerned in the retraction of newly formed blood-clots. I have found that successive reductions, obtained by centrifuging, in the platelet content of plasma result in corresponding reductions in the retraction of clots subsequently formed. This corresponds with the usual finding in clinical conditions in which there is thrombocytopenia, but several observers have reported that the platelets may be reduced without a reduction in retraction (Mackay, 1931; Bonciu, 1925; Rosenthal, 1928; Kaznelson, 1919). Apart from these functions in connexion with clotting and retraction, the platelets do not appear to be vitally concerned in the arrest of haemorrhage. There is great reluctance to abandon the original view, and in consequence attempts have been made to explain the lack of correlation between the number of platelets and the tendency to bleed by suggesting qualitative defects in the platelets (or even in the capillary endothelium) which are supposed to prevent their agglutination. But it must not be forgotten that the main basis for postulating the haemostatic importance of the platelets is, in fact, the supposedly close correlation between their number and the tendency to bleed, and it is illogical to invent additional hypotheses in support of this postulate when its basis is found wanting. It would be more interesting, and possibly more advantageous, to assume that the platelets have no part in the normal control of capillary bleeding (except in clotting and retraction), and to see where such an assumption leads. It has already been pointed out that the clotting of the blood is of little importance in the control of bleeding from small wounds, and if the platelets are also excluded, what factor remains that is capable of producing haemostasis? Only the action of the vessels themselves, a factor that has been almost completely ignored from the point of view of a possible haemostatic function.

*The vessels. Capillary contractility.* For a considerable time the observed variation in the diameter of the capillaries and their appearance and dis-

appearance was attributed to changes in the blood-pressure brought about by contraction or dilatation of the arterioles. Lister, in 1858, observed that the capillaries of the web of the frog's foot became enormously dilated during inflammation, but he thought that this change was a passive one, and due to dilatation of the vessels supplying them. In 1865 Stricker observed the contraction of single capillaries, and in 1879 Roy and Brown showed that there was actually little relation between the pressure in the capillaries and their diameter. Steinach and Kahn (1903) were able to produce contraction of capillaries by suitable stimulation, and showed that the response varied with the strength of the stimulus. Krogh (1919) showed that stimulation of muscle enormously increased the number of visible capillaries, many previously contracted vessels becoming dilated. He showed that the contracted state of the capillaries was not due to passive collapse, but was due to active constriction, since a pressure of 30 mm. of mercury did not cause them to dilate.

Though the active contraction of the capillaries is now generally recognized, the motor elements that produce it are still a subject of controversy. Rouget (1874, 1879) held the view that the stellate cells he observed reinforcing the capillary walls were contractile. This explanation has been upheld by Vimtrup (1922) and Krogh (1929). Busch (quoted by Krogh, 1929) claimed that these cells were supplied with nerve-fibres derived from the sympathetic system, and there is evidence that capillary contraction follows sympathetic stimulation (Hooker, 1920; Leriche and Policard, 1921; Harris and Marvin, 1927). Other authorities, however, deny that the Rouget cells can be the motor elements (Clark and Clark, 1925*a*, 1925*b*, 1935; Florey and Carleton, 1926; Jones, 1936). The capillaries may also be stimulated to contract directly, without the apparent involvement of a nervous mechanism. The most familiar instance of this is the 'white reaction' of Cotton, Slade, and Lewis (1917). They found that if the skin was lightly stroked with a blunt point, an area of blanching appeared after a few seconds, and persisted for several minutes. This blanching, due to emptying of the capillaries, could be obtained when the circulation in a limb had been completely arrested. Their conclusion was that the reaction is due to active contraction of the capillaries in the stroked area. Ebbecke (1917) found that the reaction could be obtained in skin that had been anaesthetized with novocain, or in which the nerve supply had degenerated. Certain substances, such as adrenalin (Cotton, Slade, and Lewis, 1917; Heimberger, 1925) and pituitrin (Carrier, 1922) also appear to cause contraction of the capillaries by direct action.

*Capillary dilatation.* The reactions involved in dilatation of the capillaries are more complicated. Dilatation may be active, or passive following an increase in pressure such as that produced by arteriolar dilatation. Active dilatation of the capillaries in the skin has been shown to follow stimulation of the peripheral cut ends of the sensory nerves supplying that area. This so-called 'antidromic' stimulation was observed by Bayliss (1901) and Doi (1920), though no connexion could be found between the capillaries and the

nerve-endings. Lewis, in a series of exhaustive experiments, provided the explanation, showing that such stimulation caused a histamine-like substance to be released from the nerve-endings, which diffused through the tissues and caused a dilatation of the adjacent capillaries (Lewis, 1927 *b*; Lewis and Marvin, 1926, 1927; Lewis, Grant, and Harris, 1927). A similar substance is also released from the tissues by trauma, heat, cold, and other irritative factors (Lewis, 1924 *b*, 1927 *a*, 1927 *b*; Lewis and Grant, 1924, 1926; Lewis and Love, 1926; Lewis and Zotterman, 1926; Lewis and Harmer, 1927; Lewis, Grant, and Marvin, 1927). If the skin be stroked firmly with a blunt point, the white reaction is not obtained, and instead there appears a red line of dilated capillaries, becoming surrounded by a spreading flare. The red line is due to the dilator effect of 'H' substance, which is therefore capable of overcoming the stimulus to contract.

*The reaction of capillaries to injury.* From the point of view of the haemostatic system, the reaction of torn or incised capillaries is of particular interest, but there is very little available information. Magnus (1924), by means of micro-incision, found that capillaries in the frog's foot and in human skin normally disappeared after division and remained invisible. Von Bernuth (1925) examined the reaction of the capillaries in cases of purpura haemorrhagica, finding that they disappeared in the normal way, but Leschke and Wittkower (1926) stated that in this condition the reaction was absent. Mackay (1931) regarded the ability of the vessel walls to contract as the all-important factor in the control of capillary bleeding.

In spite of these observations the contractility of injured capillaries is not generally regarded as of haemostatic importance, and, indeed, is seldom mentioned in this connexion. Finding it difficult to accept this position, I studied the reaction of the capillaries to injury in normal persons and in several cases of haemorrhagic diatheses. The capillaries at the base of the finger-nails were observed through a binocular dissecting microscope, the subject's hand being steadied by a special clamp. Photographs were taken, when the conditions made it possible, by means of a small camera attached to one of the oculars. Injury to a selected vessel was inflicted by the insertion of a glass fibre mounted in a mechanical manipulator. It was not always possible to observe the result, this being sometimes obscured by the haemorrhage, or by the optical disturbance caused by insertion of the needle.

In normal subjects it was found that the usual response to puncture of a capillary (when this was clearly seen) was haemorrhage lasting for a few seconds, and then the disappearance of the vessel. After a period of from 20 min. to 2 hr., the affected capillary reappeared, though the circulation was not always re-established. Plate 1, Figures 1 *a* and 1 *b*, show the first reaction, and Plate 1, Figures 2 *a* and 2 *b* the subsequent dilatation. Occasionally the capillary disappeared without any loss of blood, as in the case illustrated by Plate 1, Figures 3 *a* and 3 *b*. Twenty normal subjects were examined in this way. In some the capillary did not vanish completely after puncture, but showed one or more constrictions, with arrest of the circulation.

In others, though the vessel apparently disappeared in the usual way, on closer examination it could be seen that a few red cells were finding their way through the lumen, though this seemed to be greatly constricted. In two cases the capillaries did not definitely respond to puncture. In one, the vessel vanished after injury, but reappeared a few seconds later; in the other, the circulation of blood continued unchanged after puncture, though other vessels in the same subject reacted in the usual way. In all the cases in which disappearance occurred the capillaries subsequently became visible.

The normal result of injury, then, is the temporary disappearance of the capillaries, due presumably to the fact that they have been emptied of visible red cells. This emptying may be due to constriction of the vessel itself, to contraction of the arteriole or artery supplying it, or to the phenomenon described by Krogh (1929) as 'plasma-skimming', that is, the receipt of clear plasma instead of whole blood by the capillary, derived from the peripheral stream in the artery at the point at which the supplying arteriole is given off. It is unlikely that the capillaries were merely passively collapsed after the contraction of their arterioles, since they disappeared after puncture when a venous back pressure of 30 mm. of mercury had been obtained by the application of a tourniquet to the arm. The disappearance of capillaries adjacent to the one punctured was never seen, a point suggesting that the arterioles are not concerned, since it is probable that a single arteriole sometimes supplies more than one capillary, and for the same reason plasma-skimming is not a likely explanation of the disappearance. The available evidence is therefore in favour of the view that the emptying of injured capillaries is due to their active contraction. There is no information at the moment as to the mechanism of this reaction. The response of the capillaries to injury in the haemorrhagic states was investigated, and the findings will be discussed when these conditions are considered.

*The capillaries and haemostasis.* It cannot be asserted that because the capillaries in a certain area normally react in a certain way to puncture that all skin capillaries normally contract after any trauma that breaks their continuity, but the possibility that this is the case must be considered. There is evidence, circumstantial but nevertheless persuasive, that this reaction may have an important part in the normal haemostatic system. In the first place, unlike the other factors that have been discussed, the contraction of injured capillaries would seem to be easily capable of arresting a flow of blood. As regards the time required, though the actual process of contraction is almost instantaneous, there may be a short interval between the stimulus and the moment of contraction. In the case of the puncture of a single capillary by an extremely fine needle the damage to tissue is very small, and the local production of 'H' substance probably very slight. In the case of the much greater tissue damage inflicted during the estimation of the bleeding-time, the larger amount of 'H' substance liberated may well delay the contraction of the injured vessels until it is removed by the flow of blood or by diffusion.

under investigation all prolong the bleeding-time and produce capillary dilatation. The application of lung extract, which is a powerful blood coagulant, does not shorten the bleeding-time, but increases it fourfold, an effect that might be explained by the action of its histamine content on the contractility of the capillaries. Pituitrin, known to have a constricting effect on the capillaries, reduces the bleeding-time by a small amount only, suggesting that the vessels under examination are already being maximally stimulated to contract.

### *A Hypothesis of Haemostasis*

From what has been said, a tentative hypothesis of haemostasis can be formulated. The coagulation of the blood seems to be essential to the maintenance of haemostasis in wounds larger than those produced by a needle, but it is apparently not concerned in the initial arrest of bleeding. The platelets, apart from their function as accelerators of coagulation and retraction, apparently have no important part in the production or maintenance of haemostasis. There is no conclusive evidence that the capillaries are vitally concerned, but it has been shown that, in the case of normal subjects, they contract when punctured, and that this contraction is followed by dilatation. Other evidence has been reviewed suggesting that the arrest of bleeding from small wounds is closely related to the contractility of the capillaries concerned. It is suggested, therefore, that the probable sequence of events in the normal mechanism of haemostasis is as follows:

1. When a wound is first inflicted, bleeding occurs from the injured capillaries, dilated by the influence of 'H' substance set free from the damaged tissues.

2. When the 'H' substance has been removed by the flow of blood or by diffusion, the capillaries are able to contract and bleeding ceases, the action of the vessels being possibly assisted by the agglutination of platelets, though this factor is not essential.

3. The blood that has escaped during the interval has time to clot in the wound, and the clot becomes firmly attached and toughened by red blood cells.

4. After the period of capillary contraction, redilatation of the vessels takes place, an event that is possibly the first stage of the preformed blood-clot is now essential for the maintenance of haemostasis, since it prevents the onset of bleeding from the severed vessels.

5. In the case of punctured wounds, the presence of the clot is necessary, since the edges are in close approximation and sealed together during the period of capillary constriction to prevent the exudate.

This hypothesis will now be considered in relation to the clinical manifestations in the various haemorrhagic states.

*The Haemorrhagic States*

*Haemophilia.* This condition is aptly defined by Bulloch and Fildes (1911) as 'the inherited tendency for males to bleed', and its sex-linked inheritance is one of its most characteristic features. Its occurrence in the female has never been proved, though similar conditions have been described (Joules and Macfarlane, 1938). In haemophilia, the haemorrhage may occur from or into any part of the body. In addition to free bleeding from the surface, and from the mucous membranes of the gastro-intestinal and urinary tract, effusions of blood occur into the subcutaneous tissues, into muscles, into the nervous system, into the serous cavities, and, characteristically, into the joints. These haemorrhages usually follow definite trauma, and there is no reason to suppose that they are more easily induced than in the normal person, though the duration of the bleeding is out of all proportion to the initial damage. The onset of the bleeding may be some time after the injury. I have seen several hours elapse between the extraction of a tooth and the beginning of the uncontrollable haemorrhage which followed it, and in several cases a similar interval is a feature in the histories of accidental injury.

Apart from the direct effects of persistent bleeding, the only abnormalities that can be demonstrated are delay in the coagulation of the blood and the soft consistency of the clots when formed. The clinical severity of the case is often not proportional to the prolongation of the clotting-time. For instance, one of the cases I have been able to study was severely affected, and in two years was admitted on nine occasions to hospital, where he spent a total of seven months. Another had scarcely a day's illness and was constantly at work, yet the clotting-time of his blood was usually double that of the first patient. It is not necessary to enter into the discussion that has for many years revolved round the question of the clotting defect in haemophilia; it need only be said that, while almost every known factor has been considered to be involved and has been subsequently exonerated, up to the present no satisfactory explanation has been found. The bleeding-time is typically normal in this condition. The platelets are present in normal numbers, and seem to have no obvious qualitative defect (Eagle, 1935; Patek and Stetson, 1936), though such a defect had been previously postulated (Minot and Lee, 1916). The capillary resistance tests reveal no abnormal fragility of the vessels. Three typical cases were examined with regard to the reaction of the capillaries to puncture. The vessels in the nail-folds were found to be regular in shape, and in two cases definite contraction after puncture was observed, the response in the third not being clearly seen. Unfortunately, good photographs could not be obtained. The attempts to control the persistent bleeding in haemophilia provide interesting information. There is always a temptation to apply pressure to the bleeding-point, but if this is done haemorrhage almost invariably restarts as soon as it is released. The prolonged application of pressure is often

responsible for an extension of the bleeding area through the devitalization and sloughing of the tissues (Macfarlane, 1935). Similar unfortunate results are obtained by the use of tight plugging, or if the edges of a wound are sutured over a bleeding-point with subsequent haematoma formation. The local application of a powerful coagulant, such as that present in Russell's Viper venom, is frequently successful (Macfarlane and Barnett, 1934; Macfarlane, 1935; Baker and Gibson, 1936), but even then it may be seen that though the blood is clotted in 15 or 20 sec., an actual flow of blood is not easily stopped. The best results are obtained if the coagulant is applied on a dressing and the bleeding is arrested by moderate pressure kept up for about 5 min. When pressure is released, it is usually found that a firm clot which is capable of maintaining haemostasis has formed in the interval.

*The hypothesis applied to haemophilia.* The findings in haemophilia can be explained in terms of the hypothesis as follows: The capillaries contract in the normal way when injured, consequently the bleeding-time is normal, and there is no tendency to bleed from small punctured wounds. After injury, a short period of haemostasis may occur because of this capillary contraction. During the period of capillary contraction, however, a firm clot is not formed, so that when dilatation of the vessels takes place bleeding begins and is then persistent. The lack of solidity of the clot is therefore an important factor, and the length of the coagulation-time may not be closely related to the clinical severity of the condition. Since the haemostatic defect occurs in the blood, it is natural that the liability to bleed affects any tissue subject to trauma, and the haemorrhages are therefore distributed throughout the body.

*The haemorrhagic purpuras.* The purpuras are characterized by a tendency to bleed easily or even spontaneously, particularly into the skin or the mucous membranes, an abnormality that is clearly due to an increased fragility or permeability of the capillaries. In the haemorrhagic purpuras there is in addition a defect of the haemostatic mechanism resulting in persistent bleeding from minute injuries, or from apparently intact mucous membranes. From the point of view of the haemostatic breakdown, the haemorrhagic purpuras are very similar, but on aetiological and pathological grounds they have been divided into several groups. They are usually classified as symptomatic or idiopathic, and again as thrombocytopenic or athrombocytopenic.

Symptomatic thrombocytopenic purpura is the most common (Fowler, 1936). It may follow the administration of certain drugs to which the patient is sensitive, such as iodine (Dennig, 1933), bismuth (Bianchi, 1932), ergot and quinine (Peshkin and Miller, 1934), and particularly 'sedormid' (Boas and Erf, 1936). It may complicate acute infections, such as diphtheria, scarlet fever, typhoid, or influenza, while in measles and small-pox it gives rise to the well-known haemorrhagic forms of these diseases (Morrish, 1936; Perlman, 1934; Gram, 1920*b*; Hartmann, 1928; Arndt, 1921). It sometimes accompanies pregnancy (Rushmore, 1925), and may be associated



with menstruation (Minot, 1936). It occurs in the final stages of conditions that involve the bone-marrow, such as the reticuloses, aplastic anaemia, and carcinomatosis, frequently being the cause of death. Thrombocytopenic purpura also occurs without apparent cause, and this idiopathic form usually makes its appearance in childhood, while it may occasionally be congenital (Rushmore, 1925) or even hereditary (Witts, 1932). Clinically there is little to distinguish the two varieties. Purpura is, of course, the typical manifestation, taking the form of minute petechiae or extensive ecchymoses. It occurs most often in the skin of the limbs, but it may be found on almost any part of the body surface, particularly at points subjected to pressure or constriction. The mucous membranes may be similarly affected, and in addition there may be free bleeding, particularly from the mouth or the nose, which is apparently unconnected with trauma. Any injury, even the minute puncture made by a hypodermic needle, may ooze blood for hours. Though subcutaneous haemorrhage may sometimes occur, it is rare for the massive effusions of blood into the deep tissues so often seen in haemophilia to take place, while bleeding into the joints is practically unknown. In certain cases this restriction of the haemorrhage to certain parts may be even more marked. In women menorrhagia may be the first, and sometimes the only, symptom of thrombocytopenic purpura (Fowler, 1936; Hartfall and Oldfield, 1934; Israel and Mendell, 1939). In other cases epistaxis, or bleeding from the gastro-intestinal tract, may be the main feature of the disease. The tendency to persistent bleeding may even vary from one part of the skin to another, as in the case described by Roskam (1922) in which the bleeding-time from the left ear was 3 min., and from the right 48 min.

The reduction in the platelet count is very variable. Certain so-called 'critical levels' have been defined, below which haemorrhage is supposed to occur. Duke (1912) puts this level at 40,000 platelets per c.mm., Frank (1925) at 30,000, and Gram (1920*a*) at from 100,000 to 200,000, so that no close agreement exists. Even the day-to-day variations in the same individual are not closely correlated with the clinical state. The coagulation time in the thrombocytopenic purpuras is typically normal, and the clot formed *in vitro* is firm. Clot retraction, however, is usually reduced, or even absent, and follows the platelet count closely. Apart from the shortage of platelets, and the anaemia subsequent to haemorrhage, there is no characteristic blood picture. The capillary resistance is reduced, and the application of a tourniquet may result in extensive capillary bleeding in the distal portion of the limb constricted.

The reaction of the nail-fold capillaries to puncture was investigated in four cases of idiopathic thrombocytopenic purpura and in one case of symptomatic thrombocytopenic purpura associated with aplastic anaemia. In all, the bleeding-time was greatly prolonged, and the platelet counts ranged from 22,000 to 80,000 per c.mm. In the idiopathic cases the capillaries were of very irregular and distorted forms, sometimes branching.

These vessels were found to remain visible after puncture, sometimes with free bleeding from the needle-track for several minutes (Plate 2, Figures 4a, 4b, 5a, and 5b). In one case it was observed that the more regularly shaped vessels contracted after injury, though misshapen ones in the vicinity did not. In the case of the symptomatic thrombocytopenia, the capillaries were regular but failed to react to puncture.

*Athrombocytopenic purpura.* This is a condition which resembles the more familiar thrombocytopenic variety of purpura in its manifestations. It is usually hereditary, affects males and females, and is passed from an affected subject to his or her children. Over 60 cases have been described, and there are records of about 90 affected relatives not investigated in detail. The condition is not well recognized because it has been described under several different names, and only a comparison of the findings reveals the fact that these probably refer to one and the same condition, though the demarcation between this and other haemorrhagic states is not always well defined. Von Willebrand (1931), describing a large group of cases among the inhabitants of the Åland Islands, called the condition 'hereditary pseudohaemophilia', though there is little clinical, pathological, or genetic resemblance to haemophilia. It has also been called Von Willebrand's disease, Glantzmann's disease, and 'hemogenia'. Glantzmann (1918) himself described a condition that he called 'hereditary haemorrhagic thromboasthenia', and some of his cases were apparently examples of athrombocytopenic purpura.

Clinically these cases are almost indistinguishable from the thrombocytopenic variety. The bleeding tendency is as severe, and several fatal cases have been recorded. The capillary resistance may be reduced, the bleeding-time is greatly prolonged, and the clotting-time is usually normal, but in some instances it is slightly prolonged. There is some variation in the findings with regard to the clot retraction. Glantzmann stated that in his cases it was reduced or absent and postulated a platelet defect to explain this finding. Out of nineteen instances in the literature in which this investigation was made, however, only four were reported to have deficient retraction, and in the remainder it was stated to be normal.<sup>1</sup>

It has been suggested that the bleeding in this condition, if it cannot be explained by thrombocytopenia, must be due to a qualitative defect in the platelets which prevents their agglutination in contact with foreign surfaces. Morawitz and Jürgens (1930) and Von Willebrand and Jürgens (1933) using an apparatus called a 'capillarthrombometer' claim to have shown that the platelets in such cases do not agglutinate as rapidly as normal ones. The conditions of this estimation, however, are very different from those in the living subject, and even if this slowness of agglutination can be confirmed, it does not necessarily explain the haemorrhagic tendency. Best, Cowan,

<sup>1</sup> Buckman, 1925; Giffin, 1928; Kennedy, 1928; Minot, 1928; Rosenthal, 1928; Rothman and Nixon, 1929; Kugelmass, 1932; Farber, 1934; Bailey and McAlpine, 1935; Fowler, 1937; Mathewson and Cameron, 1937; Schlicke and Hall, 1938; Bain, 1939.

and Maclean (1938) have shown that heparin injected into the blood-stream of animals will prevent the agglutination of platelets on glass and cellophane tubes introduced into the circulation, but in my own experience heparin given experimentally to animals or therapeutically to human subjects in sufficient amounts to render blood-samples permanently fluid does not prolong the bleeding-time.

I have been able to investigate five patients with this condition, all females, and the findings are tabulated in the table below.

It will be seen that the clotting-times in two cases are slightly prolonged, and that in one case the clot retraction is below the lower normal limit of 44 per cent. The method used for the latter estimation has been described previously (Macfarlane, 1939). The platelets were of normal morphology in these cases, and could be seen to clump actively in contact with glass.

Case	A	B	C	D	E
Platelets, per c.mm.	260,000	395,000	320,000	352,000	385,000
Bleeding-time	over 1 hr.	24 min.	over 2 hr.	7½ min.	over 20 min.
Clotting-time, in min. (Lee and White's method, except in case B, which was by Dale and Laid- law's method)	9	2¼	8	14	12
Clot retraction, per cent.	46	34	56	56	60
Capillary resistance test	negative	positive	positive	positive	negative
Members of family affected	none	2 male 2 female	11 male 11 female	(Mother of C)	(Aunt of C)

The capillaries were studied with regard to reaction to puncture. They were of distorted and often bizarre forms, and did not contract after injury in any instance (Plates 2 and 3, Figure 6 *a, b, c*, and *d*; Figure 7 *a, b*).

*The treatment of the purpuras.* The reaction of the purpuras to treatment is instructive. In the case of the symptomatic purpuras, of course, it is the primary condition that has to be dealt with. As regards the idiopathic type of thrombocytopenic purpura, Vaughan (1937) has reviewed the various therapeutic procedures which have been advocated and points out that their very multiplicity suggests that none are specific.

The only form of treatment that has been widely applied with success is splenectomy, which gives good results in over 70 per cent. of cases. This operation was advocated in 1919 by Kaznelson, on the supposition that the abnormal spleen commonly found in this condition was destroying excessive numbers of platelets and so was responsible for the bleeding. Though in some cases the hypothetical reasons for the operation seemed to be well founded in that the result was a rise in the platelet count accompanied by clinical improvement, it soon became apparent that the effects were not so simple as originally supposed. In the first place, clinical improvement may occur immediately after operation, before an increase in the number of platelets can have taken place. Clopton (1925), for instance, observed in

two cases that abnormal bleeding from the operation wound, and epistaxis which had persisted prior to the operation, ceased as soon as the spleen was removed, while the patients were still on the operating-table. In these cases the bleeding-times were reduced to 1 min. and 2 min. immediately after operation, though the platelets did not rise significantly for several days. Brill and Rosenthal (1923) recorded two similar cases and reported that in one the platelets fell to 3,000 per c.mm. after splenectomy and the bleeding-time to 2 min. In the case described by Jennings and Castleden (1939) frequent estimations of the platelets, bleeding-time, and capillary fragility revealed the fact that, after splenectomy, the platelets did not rise above their pre-operation level until five days had passed, though on the third day the bleeding-time had fallen to four minutes and the capillary resistance test was normal. In some instances there is a cure of the haemorrhagic condition without a rise in the platelet count at any time. Spence (1928), reviewing the results of splenectomy in 101 cases, found that in 17 there was no increase in the number of platelets after operation, but that in seven of these the haemorrhagic condition was cured, and in two others it was improved. More often the platelets increase, but subsequently return to their previous low level. Out of 26 cases followed after operation, the platelets returned to a low figure in 16, but of these only three showed a corresponding relapse in the haemorrhagic condition, and in 11 there was an apparently permanent cure. Spence quotes the following corresponding estimations of the bleeding-times and platelet counts in some of these cases:

Platelets, per c.mm.	Bleeding-time, in minutes
60,000	1
12,000	2
31,000	3
12,000	3
20,000	2
120,000	2
109,000	3
20,000	$\frac{1}{2}$
80,000	3
100,000	$1\frac{1}{2}$

Conversely, the platelet count may rise to normal levels after the operation though the haemorrhagic symptoms may persist. Clopton (1925) reports such a case, in which the bleeding-time was 16 min. though the platelets had risen to 200,000 per c.mm. Spence includes four similar instances in his review, and in one the platelet count was 284,000 per c.mm. though the bleeding-time was 33 min. Marzullo (1933) quotes three further cases.

It is clear that the haemorrhagic tendency usually ascribed to thrombocytopenia may be present without a reduction in the platelets, and that this tendency may be absent when the platelets are greatly reduced. It is true, however, that a reduction in the platelets is often associated with a tendency to abnormal capillary bleeding, and it is suggested that there may be some factor which is responsible both for the thrombocytopenia and the condition of the capillaries. The effect of splenectomy is particularly interesting.

The immediate clinical result sometimes seen cannot be explained on the basis of an alteration in the number of circulating platelets. It might be due to the sudden restoration of capillary function, though the mechanism, nervous or chemical, by which this is brought about is unknown. The nature of the capillary defect in the purpuras, and its possible relation to the spleen and to the production of the platelets, are matters only for speculation at the present time, but in this connexion it is of interest to remember Bedson's (1922) observations on the effect of anti-platelet serum on splenectomized animals. The observation of Laplane and Brocard (1937) that experimental irritation of the sympathetic nervous system produced lesions of the capillaries and haemorrhage may also have a bearing on the aetiology of the purpuras.

In the athrombocytopenic form of purpura the results of splenectomy are not quite so good. Five instances are recorded in which this operation was carried out. Three of these were improved, one bled to death, and the remaining one was unaffected (Little and Ayres, 1928; Kugelmass, 1932; Kennedy, 1928; Giffin, 1928).

As regards the local treatment of the capillary bleeding from the mucous membranes or small injuries, the application of coagulants is not very effective in the purpuras. It will usually be found, however, that pressure applied for about five minutes to the bleeding-point will result in haemostasis which is maintained after the pressure is released, in contradistinction to haemophilia in which pressure is effective only during its actual application.

*The hypothesis applied to the purpuras.* From the point of view of the hypothesis of haemostasis, the findings in the purpuras would be explained as follows. The essential haemostatic defect is the inability of the capillaries to contract after injury. Thus the bleeding-time is prolonged because the initial flow of blood remains unchecked, and the formation of a firm clot in contact with the bleeding-point is impossible. Coagulants are not effective, because even rapid clotting of the issuing blood may be unable to arrest the haemorrhage if the flow is continuous. Pressure is effective because this arrests the flow and gives time for the formation of a firm clot that can maintain haemostasis when the pressure is released. The capillaries, in addition to their functional defect, are also more fragile than normal, so that haemorrhage is easily induced. This assumed increased fragility may be connected with their failure to contract after injury.

On the basis of an essential capillary defect in purpura it is possible to explain the localization of the haemorrhage. If the persistent bleeding were due to the lack of platelets, the liability to bleed should be equally distributed. If it be supposed, however, that the capillaries are at fault, and that it is mainly the vessels of the skin and the mucous membranes that are affected, the localization becomes understandable. In some cases the vessels of a certain part may be particularly affected. The fact that bleeding into the deep tissues is rare and into the joints is almost unknown might be explained by supposing that the vessels in these situations are functionally

normal. It is possible that the defective clot retraction in the thrombocytopenic variety of purpura may reduce the haemostatic efficiency of the clot. That this factor is not the cause of the haemorrhagic tendency is shown by the fact that severe bleeding occurs in the cases of athrombocytopenic purpura in which clot retraction is normal.

*The haemorrhagic tendency in jaundice.* The haemostatic defect occurring in some patients with jaundice is more complicated than in the conditions previously discussed. As long ago as 1894 Robson pointed out that such patients were liable to uncontrollable bleeding after operation. Colbeck (1932) and Wangensteen (1928) published figures suggesting that post-operative bleeding is one of the most important causes of the operative mortality in jaundice. McNealy, Shapiro, and Melnick (1935), who have observed a large series of cases, stated that there was usually little difficulty in obtaining haemostasis at the time of operation and that bleeding did not begin for some hours afterwards.

The defect in the haemostatic mechanism in haemorrhagic jaundice is not easy to locate. There is sometimes a well-defined delay in the clotting-time of the blood, and for many years this was attributed to an alteration in the calcium available for the process of coagulation (Wright and Paramore, 1905; Lee and Vincent, 1915; Walters, 1932). Other workers, however, established that this supposed abnormality had very little to do with the haemorrhagic tendency (Ivy, 1930; Snell and Greene, 1930; Colbeck, 1932), and that the administration of calcium had little or no therapeutic value (Wangensteen, 1928; Ravdin, Riegel, and Morrison, 1930). Attention was then directed to the prolonged bleeding-time that is also observed in some, but not all, of these cases. Ivy, Shapiro, and Melnick (1935) devised a modified method of estimation employing a venous back pressure of 40 mm. of mercury in the arm in which the test was made, a procedure that does not normally prolong the bleeding-time. Using this method, McNealy, Shapiro, and Melnick (1935) investigated a group of 810 jaundiced patients, and they claimed that an increased bleeding-time by this method was obtained in a large proportion of the patients who exhibited haemorrhagic tendencies.

More recently the problem of coagulation defect has again been raised. In 1924 Murakami and Yamaguchi had remarked on the decreased thrombin production that they found in blood from cases of jaundice, and Lewisohn (1931) reported a similar decrease in the prothrombin content. Hawkins and Whipple (1935) and Hawkins and Brinkhous (1936) stated that the haemorrhagic tendency developed by dogs in which bile fistulae had been established was due to a prothrombin deficiency, and could be made good by adding bile to the diet. This observation could be linked with the haemorrhagic condition in chicks thought by Dam (1934, 1935) to be due to a prothrombin deficiency following the absence from the diet of a specific fat-soluble vitamin. Since Heymann (1937) has shown that fat-soluble vitamins are not absorbed in the absence of bile-salts, the supposed reduction of prothrombin in cases of obstructive jaundice also, suggested by Quick, Stanley-Brown, and Bancroft

(1935), may well be due to the non-absorption of the necessary factor now known as vitamin K. This is substantiated by the work of Butt, Snell, and Osterberg (1938) and Scanlon, Brinkhous, Warner, Smith, and Flynn (1939) who claim to have reduced the 'prothrombin time' in cases of jaundice by giving vitamin K.

It is probable that a marked deficiency of prothrombin may exist in the blood without an increase in the coagulation time as usually measured. Such a deficiency, however, may well result in the formation of friable clots, and it has been remarked by McNealy, Shapiro, and Melnick (1935) that such clots are a feature in jaundice. There is also in some cases a deficiency in clot retraction in this condition, but this is not always correlated with the liability to bleed (Macfarlane, 1939). In jaundice, therefore, there is a defect in the clotting of the blood, probably due to a deficiency of prothrombin. There is also a defect in the control of bleeding from small wounds which is accentuated when the capillary blood-pressure is raised to a degree that would have no effect on the normal mechanism. It is suggested that the haemorrhagic tendency is due in part to an impairment of the contractile power of the capillaries that may be revealed only when the added burden of increased pressure is placed upon them, and in part to a clotting defect that results in the formation of friable clots. The fact already noted, that bleeding does not as a rule take place for some time after injury, suggests that in these cases the capillaries are contracting normally, and it is the instability of the clot that is the cause of the bleeding subsequent to capillary dilatation.

*Haemorrhagic disease of the new-born.* This condition occurs in about one of every 400 new-born babies. Haemorrhage occurs from the mucous membranes, particularly of the gastro-intestinal tract, and from the umbilicus. The onset is described by Capon (1937, 1932, 1924) as dramatically sudden, in an otherwise healthy child, and in about 8 per cent. of cases the bleeding is fatal. Both Rodda (1920) and Beveridge (1928) found a prolongation of the clotting-time and the bleeding-time in the cases they observed. In this connexion it should be pointed out that the normal coagulation time in infants is increased by 30 to 50 per cent. as compared with the adult. This may be explained by the observation of Brinkhous, Smith, and Warner (1937) that in the first few weeks of life the prothrombin content of the blood is only about 14 per cent. of the adult level. These workers found that in a case of the haemorrhagic condition this prothrombin content was even lower, being less than 5 per cent. Waddell, Guerrey, Bray, and Kelley (1939) have confirmed these findings, and claim to have reduced the prothrombin times in infants by giving vitamin K. Thus the haemorrhagic disease of the new-born is very similar to that found in jaundice, and the failure of the haemostatic mechanism may be explainable on the same basis.

*Hereditary haemorrhagic telangiectasia.* This condition is a clear-cut example of a haemorrhagic tendency that can be attributed only to a purely vascular defect. The essential lesion is the telangiectasis, a group of dilated capillaries in the skin or the mucous membranes. Profuse or even fatal bleeding may

take place from such vessels if they are ruptured. The condition is hereditary, affects males and females, and is transmitted from affected persons to their children. The telangiectases are never present at birth (Hurst and Plummer, 1932) and usually make their appearance in late childhood. The histology of the lesions was investigated by Hanes (1909), who observed that they were composed of blood-vessels formed of a single layer of endothelium with no demonstrable muscle or connective tissue in their walls. Lewis (1926) found that they did not react to stimuli which affect normal capillaries. The telangiectases are found most often on the mucous membranes of the nose and in the mouth. They are also found in the stomach (Osler, 1901, 1907; Boston, 1930), in the rectum (Hurst and Plummer, 1932), and in the bronchi (Fitz-Hugh, 1931). In the skin they occur most frequently on the lips and cheeks, and also on the tips of the fingers and under the nails.

Haemorrhage is usually in the form of epistaxis, and may follow slight trauma such as blowing the nose, or an increase in the blood-pressure such as is produced by stooping. Bleeding from the lesions in any part of the body may be very persistent, and at least five fatal cases of epistaxis have been reported. In the case of the telangiectases in the skin, bleeding may follow injury of the affected vessels, as after scrubbing the hands (Foggie, 1928; Hurst and Plummer, 1932). No other abnormality can be found in this condition. The clotting of the blood, the platelets, and the unaffected vessels are all normal. The bleeding-time is normal if the dilated vessels are not injured. I have found, however, that if one of the affected capillaries is pricked, the bleeding-time is indefinitely prolonged, so that the haemorrhage has eventually to be arrested by pressure.

Two cases have been examined by means of the capillary microscope. The unaffected capillaries in the nail-fold contracted in the usual way after puncture. Telangiectases occurring on the fingers in these cases were examined and showed great dilatation of the capillary loops, which did not contract after puncture (Plate 3, Figures 8*a*, *b*).

The bleeding can almost always be arrested by pressure on the bleeding-point. One patient made the observation that his epistaxis could best be stopped by inflating a small rubber bag in his nose (Osler 1901), and this device has been applied with success by Hurst and Plummer (1932). From the point of view of the haemostatic mechanism the position is clear. This condition is an excellent demonstration that normal clotting of the blood cannot arrest a flow of blood, and that normal platelets in normal numbers are equally ineffective. The demonstrable abnormality is the failure of the abnormal capillaries to contract after injury. Thus the localized lesion in this state may be compared to the more widespread abnormality in the purpuras. The bleeding-time is normal from the unaffected vessels, but prolonged if the non-contractile and dilated capillaries are injured.



*Summary and Conclusions*

1. Evidence has been reviewed which suggests, to the author, the following conclusions: (a) That the platelets, apart from their role as accelerators of blood coagulation and clot retraction, do not appear to have an important part in the haemostatic mechanism. (b) Coagulation of the blood is itself incapable of arresting the bleeding even from a needle puncture. The formation of a firm blood-clot is, however, necessary for the maintenance of haemostasis produced by some other factor. (c) Capillaries, in certain areas examined, normally contract after injury, but redilate subsequently. This contraction is absent in the haemorrhagic states associated with a prolonged bleeding-time.

2. From this evidence a tentative hypothesis of haemostasis has been formulated, as follows: (a) The first normal reaction to injury is haemorrhage from damaged vessels, which are dilated by the action of 'H' substance produced by the trauma. (b) When the 'H' substance has been removed by the flow of blood, or by diffusion, the injured vessels contract, and bleeding ceases, the action of the vessels being possibly assisted by agglutinated platelets, though this factor is not essential to haemostasis. (c) During the period of capillary contraction the blood which escaped initially has time to coagulate firmly in the wound and the clot to retract and become securely attached. (d) After the period of contraction dilatation of the injured capillaries takes place. Haemorrhage, in the case of open wounds, is now prevented by the preformed blood-clot. (e) In the case of small punctured wounds the approximation of the edges and drying of the exudate is enough to prevent the onset of bleeding, and blood-clotting is not necessary for haemostasis.

3. (a) Failure of this mechanism may be due to a loss of capillary contractility, to defective blood-clotting, or to both. Such a failure is responsible for the haemorrhagic states.

In the case of defective capillary contractility, haemorrhage is persistent even from minute wounds in spite of normal coagulation. The bleeding-time is thus prolonged. The conditions in which this occurs are: (i) Thrombocytopenic purpura, in which the capillary abnormality is associated with a shortage of platelets. Splenectomy may restore the capillary function without a rise in the platelet count, or may fail, in spite of such a rise. The haemorrhagic tendency may be localized to a particular site, because the vessels in other situations react normally. (ii) Athrombocytopenic purpura, in which the capillary abnormality is not associated with a shortage of platelets. The bleeding-time is greatly prolonged, and the haemorrhagic tendency is as severe as in the thrombocytopenic variety. (iii) Haemorrhagic telangiectasia, in which there is a sharply localized abnormality of the capillaries. Those forming the lesions do not contract after injury, and it is from these that persistent bleeding occurs. The platelets and coagulation of the blood are normal. In these conditions temporary pressure is an effective local measure

because a firm blood-clot which is capable of maintaining haemostasis forms during its application.

(b) In the case of defective blood coagulation a firm clot is not formed during the interval of capillary contraction. Consequently, when dilatation of the wounded vessels occurs, the clot is incapable of withstanding the pressure, and persistent bleeding from open wounds takes place. Bleeding also may occur in any tissue liable to trauma. Pressure alone is ineffective, but substances that will coagulate the blood firmly are often successful. The bleeding-time in these conditions is normal, because capillary contraction takes place, and firm coagulation is not required for the maintenance of haemostasis in small punctured wounds. Defective coagulation is responsible for the haemorrhagic tendency in haemophilia, pseudo-haemophilia, fibrinopenia, some cases of haemorrhagic jaundice, and haemorrhagic disease of the new-born.

(c) In some conditions, notably jaundice and haemorrhagic disease of the new-born, and also in certain of the rare haemorrhagic states described under a variety of titles, there may be a combined capillary and coagulation defect.

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FIG. 1a

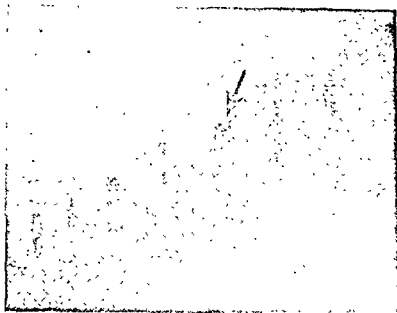


FIG. 1b

Normal capillaries, and the reaction of one of them to puncture. The arrow indicates the approximate path of the needle

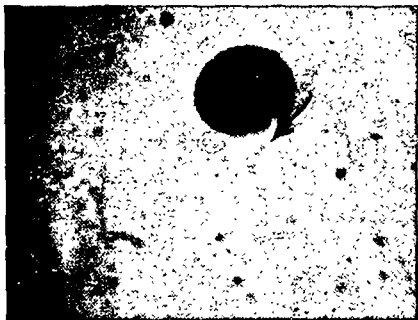


FIG. 2a. Photograph taken a few seconds after puncture of a capillary. A small amount of blood has escaped into the tissues, but the injured capillary is invisible



FIG. 2b. Photograph taken half an hour later, showing the reappearance of the capillary that had been punctured

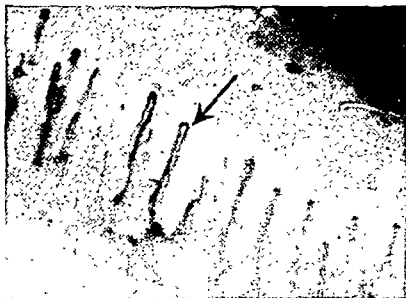


FIG. 3a



FIG. 3b

Photographs showing the immediate reaction of a normal capillary to puncture





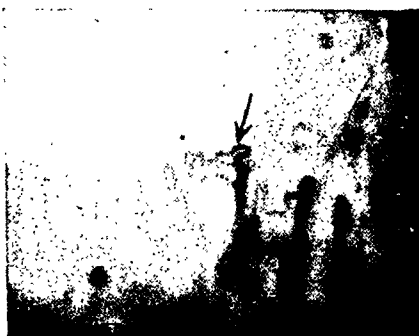


FIG. 4a

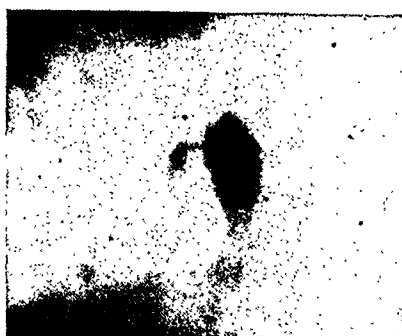


FIG. 4b

Distorted capillary in a case of chronic thrombocytopenic purpura, and the reaction to puncture

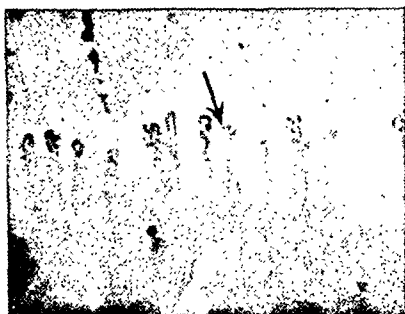


FIG. 5a

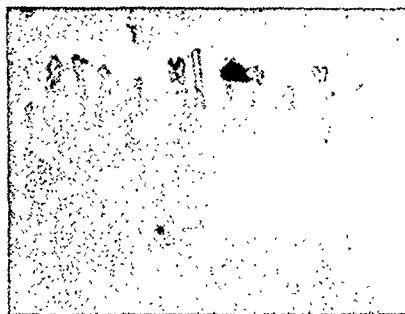


FIG. 5b

Distorted capillaries in a case of chronic thrombocytopenic purpura, and the reaction to puncture



FIG. 6a. The capillaries in a case of athrombocytopenic purpura (Case C)

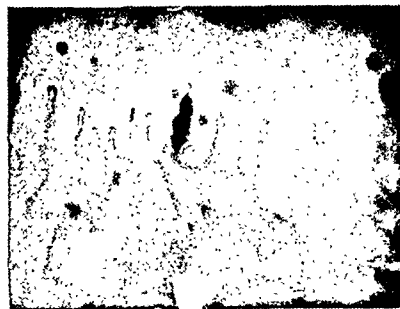


FIG. 6b. Photograph taken immediately after puncture, showing haemorrhage from the arteriolar limb





FIG. 6c. Two minutes after puncture



FIG. 6d. Five minutes after puncture. During this period blood had been escaping continuously from the needle track

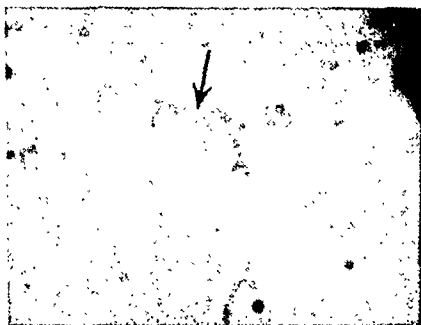


FIG. 7a. The capillaries in a case of athrombocytopenic purpura (Case A)



FIG. 7b. The reaction to puncture. Haemorrhage continued in this case also for several minutes

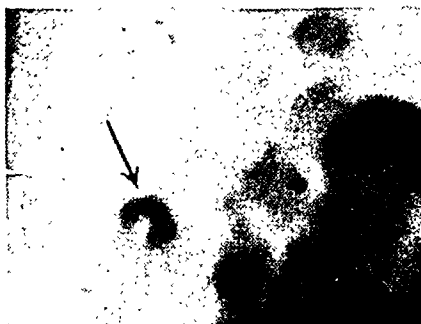


FIG. 8a. Photograph showing the greatly dilated capillary loops forming a telangiectasis

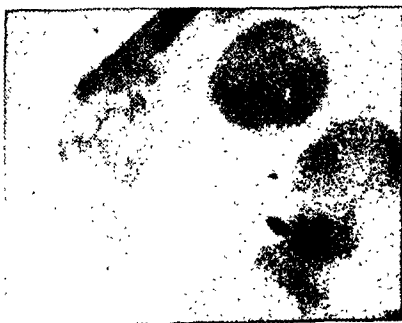


FIG. 8b. The reaction to puncture. Haemorrhage from the needle track had eventually to be arrested by pressure

the fluorescence from aneurin in each specimen of urine examined has been recognized. A full description of these modifications has been given in a recent paper (Hills, 1939). In an attempt to simplify the test for routine purposes, the response to a standard test dose as well as the content of the twenty-four hourly specimen of urine was studied, a procedure which had already been suggested by Westenbrink and Goudsmit (1937*b*). A comparison of these two methods has shown that not only is the test dose method more quickly performed, but that it is also more sensitive owing to the greater concentration of the substance in the urine.

An accurately collected twenty-four hourly specimen of urine was obtained as soon as possible after the admission of a patient to hospital. A few c.c. of concentrated HCl and 1 c.c. of toluene were added to preserve the acidity of the urine and to prevent the growth of moulds. After collection of the twenty-four hourly specimen a breakfast low in aneurin was given (e.g., white bread and butter, honey, tea). The bladder was emptied at the end of three hours, and a similar meal to the above was given supplemented by 1 mg. of aneurin by the mouth. At the end of three hours the urine was again collected. Oral administration of the test dose was preferred, as after subcutaneous injection the very high concentration in the blood allows overflow through the kidney even in cases of deficiency (Westenbrink and Goudsmit, 1938). A three-hour test period was used since the maximum response to the test dose occurs during the second hour. The patient was encouraged to take fluid during the test to ensure an adequate output of urine. The following table shows the average excretion of aneurin in twenty-four hours, and the result of the test dose in six normal controls:

Sex.	Date.	Excretion of aneurin in micrograms.		
		24-hours.	3-hours (no B <sub>1</sub> ).	3-hours (+B <sub>1</sub> ).
M.	26-27.4.39	106	6	26
M.	4-5.5.39	58	8	72
F.	3-4.4.39	50	6	28
M.	25-26.4.39	134	3	64
M.	20-21.4.39	74	6	79
M.	24-25.4.39	171	16	79
M.	3-4.4.39	99	14	109
Mean		100	8	65

The results of the twenty-four hours' excretion were roughly parallel with those obtained after the test dose, but considerable variation may occur, especially with urine of low aneurin content. The average twenty-four-hour excretion in the control cases was 100 micrograms. After a test dose the lowest figure was 26 micrograms and the highest 110 micrograms, with a mean of 65 micrograms. Duplicates are reproducible to  $\pm 10$  per cent., except for specimens containing less than 10 micrograms per three hours, when the reproducibility is  $\pm 1$  microgram per three hours, or 8 micrograms per twenty-four hours. It is doubtful, however, how far these lower values represent the true aneurin content of the sample, especially the figures below 5 micrograms per three hours. A critical account by one of us

(G. M. H.) of the sources of error has appeared elsewhere, and it suffices to state here that excessive pigmentation tends to produce high results, while a high blank fluorescence may produce low results. These sources of error seem to be associated in the same urine, so that the agreement between the thiochrome and bradycardia values may be due to a fortunate compensation of errors (Wang and Harris, 1939). In exceptional cases, the fluorescent material initially present may be destroyed on oxidation to such an extent as to mask the increase in fluorescence due to thiochrome formation. In such circumstances no value can be reported, though it seems unlikely to be high, owing to the general correspondence between the thiochrome values and the aneurin intake, especially as seen in cases undergoing treatment, some of which will be described later.

Before proceeding with the results of aneurin estimation, mention will be made of the results obtained by several workers who have studied the requirement of aneurin by the average healthy adult. Cowgill's (1934) pioneer work in this field established the fact that the requirement depended on the total calorie content of the diet and on the metabolism of the individual. Williams and Spies (1938) have adopted a somewhat different viewpoint based on the fact that an increased intake of carbohydrate raises the requirement of aneurin whilst an increased amount of fat lowers it. This can be seen from the following table, which assumes varying proportions of fat in a diet of 2,500 calories.

Table showing basis of A/NFC ratio :

Fat calories.	Non-fat calories.	Aneurin, micrograms per diem.
0	2,500	750
500	2,000	600
700	1,800	540
1,000	1,500	—

This table shows that with an increasing proportion of fat in the diet, less aneurin is required to maintain health, and that the aneurin content of the diet should be about one-third of the non-fat calories. Williams and Spies, therefore, in place of Cowgill's formula, aneurin/calories, have suggested the formula aneurin/non-fat calories (A/NFC). They estimate that the daily requirement of aneurin varies between 1 and 2 mg. (1,000 to 2,000 micrograms), but that this figure may be too low when the metabolism of the subject is raised as in fever, thyrotoxicosis, pregnancy, and heavy exertion. The majority of clinicians have used Cowgill's formula in investigating the aneurin content of patients' diets. Jolliffe, Colbert, and Joffe (1936) in America, and Alsted and Lunn (1938) in Copenhagen found that in a group of alcoholics, as long as the aneurin content of the diet was adequate, polyneuritis did not develop. Of the patients recorded in this paper it was possible in only two cases to obtain a dietetic history of approximate accuracy. The formulâ adopted was that proposed by Williams and Spies (1938).

*Results of Estimation of Aneurin in the Urine*

Ten neurological cases were investigated, the majority being examples of nutritional polyneuritis. Four of the patients gave a history of alcoholism and a deficient diet, whilst the remaining three were examples of gross under-nourishment. The following table shows the results:

Sex.	Excretion of aneurin in micrograms.			Diagnosis.
	24-hours.	3-hours (no B <sub>1</sub> ).	3-hours (+B <sub>1</sub> ).	
	100	8	65	Mean normal values
F.	8	5	6.5	Nutritional polyneuritis
F.	16	—	—	Alcoholic polyneuritis
F.	17	1	29	Nutritional polyneuritis
F.	24	4	33.5	Nutritional polyneuritis
M.	20	1	0	Alcoholic polyneuritis
M.	26	2	18	Alcoholic polyneuritis
F.	50	11.5	19	Alcoholic polyneuritis
M.	51	1	20	Subacute combined degeneration of cord
M.	88	0	45	Acute toxic polyneuritis
M.	155	6.5	102	Disseminated sclerosis

All the seven cases of nutritional polyneuritis, including the alcoholics, showed figures below the normal both for the twenty-four-hour specimen and after the test dose.

*The effect of treatment on the excretion of aneurin.* In several cases a number of estimations were made on the urine during treatment, and the following cases may be cited as examples:

T. G., male, aged 30 years, was admitted to the Courtauld Ward of the Middlesex Hospital on May 3, 1939. In February of the same year he had begun to suffer from pains in both feet, followed by stiffness, numbness, and weakness in the legs. Later he had noticed tenderness of his fingers. These symptoms had slowly become worse up to the date of admission to hospital. For a week in January 1939 he had suffered from shortness of breath on exertion, and in 1933 this symptom had been marked, and had been accompanied by a painless swelling of the legs, which disappeared only after several weeks' treatment in another hospital. For the previous 12 years he had been a heavy drinker, consuming at least a bottle of whisky daily up till 1938, when he took to beer, his average daily consumption being 12 pints.

On examination he presented the typical facial appearance of chronic alcoholism. There was no defect of memory or orientation. The pupils and cranial nerves were normal. While motor-power in the upper limbs was unimpaired, there was a moderate degree of weakness in the movements of both feet. Coarse tremor was a marked feature of all movements of the limbs. Extreme tenderness of the hands, calves, and feet was present, with hyperaesthesia to cotton-wool and pin-prick on the fingers and feet. Postural sense was normal in the fingers and toes, but vibration sense was absent in the lower limbs. The knee and ankle jerks were slightly increased. The apex beat of the heart was  $4\frac{1}{2}$  in. from the midsternal line, and there was a localized systolic murmur at the apex. The pulse-rate for the first week in hospital ranged between 100 and 110 per minute, subsequently dropping to about 80 per minute. The blood-pressure was 115/85. An

electrocardiogram was normal. There was no oedema of the ankles. The liver was slightly enlarged and firm. A fractional test meal showed a normal curve for free hydrochloric acid.

The following table shows the dietetic history:

Date.	Protein gm.	Fat gm.	Carbohydrate gm.	Beer.	Total calories.	Aneurin content.	A/NFC ratio.
Before				7 pts.			
Oct. 1938	110	140	250	(= 1,680 cal.)	4,458	974	0.3
After				4 pts.			
Oct. 1938	55	70	150	(= 960 cal.)	2,451	311	0.17

Prior to October 1938 the largest part of the total calories was provided by beer, and, although the diet was deficient in aneurin, the total amount of fat was reasonable, and sufficient to raise the A/NFC ratio to the normal figure of 0.3. However, after October 1938, although the amount of beer was less, symptoms of polyneuritis developed within three months, presumably because the total amount of fat had dropped considerably, so reducing the A/NFC ratio to the low figure of 0.17. On May 6, 10 mg. of aneurin was given subcutaneously, and this dose was continued until May 12, when the same dose was given orally. On May 26, subcutaneous injections were again resumed and continued until June 13, when the dose was diminished to 1 mg. orally. The results of this intensive treatment were slow to appear, pains and muscular tenderness being still quite marked a month after admission. Thereafter improvement took place, and on the patient's discharge from hospital in the middle of August 1939 tenderness was no longer present, power was nearly normal in the lower limbs, and only a mild hyperaesthesia remained on the dorsum of the feet.

Table showing effect of treatment on excretion of aneurin:

Date.	Aneurin treatment.	Excretion of aneurin in micrograms.		
		24-hours.	3-hours (no B <sub>1</sub> ).	3-hours (+B <sub>1</sub> ).
4-5.5.39		26	2	8
12-13.5.39	10 mg. daily from	250	25	121
22-23.5.39	6.5.39 to 13.6.39	602	57	128
15-16.6.39		500	140	190

These figures suggest that a rapid and complete saturation had taken place by the end of the first week's treatment with 10 mg. of aneurin given subcutaneously. The very high figures obtained on two subsequent occasions suggest that the continued use of 10 mg. daily was unnecessary, and that a much smaller dose, for example 3 mg. orally, would have been sufficient, especially in view of the relatively slow clinical improvement which followed the giving of the larger doses.

Aneurin when given by the mouth may lead to a saturation as marked as when it is given subcutaneously, as the following case shows:

M. N., male, aged 37 years, was admitted to the neurological ward of the Middlesex Hospital on July 30, 1939. Since 1938 he had been taking whisky and beer in excessive amounts. In March 1939 he lost his appetite, seldom taking any breakfast and eating much less at other meals than normal. At the beginning of April symptoms of polyneuritis appeared, followed by swelling of the ankles and shortness of breath on exertion.

On examination, memory and orientation were normal. The abnormal signs in the nervous system consisted of nystagmus, slight loss of power in

hands and feet, blunting to pin-prick and anaesthesia to cotton-wool as high as the elbows and knees, with hyperaesthesia of fingers and soles of feet, and tenderness on pressure of muscles. The upper limb reflexes and knee jerks were present, but the ankle jerks were absent, and the plantar responses were flexor. The heart was not enlarged. The rhythm was normal and there were no added sounds. The pulse-rate varied between 90 and 100 per minute. An electrocardiogram showed an inversion of 'T' wave in all leads, most marked in leads I and II (Dr. D. E. Bedford). There was no oedema of the ankles, and the liver was not palpable. A fractional test meal showed only traces of free HCl. A blood count on July 31, 1939, showed red cells 3,810,000 per c.mm.; haemoglobin 90 per cent.; colour index 1.2; white cells 6,000 per c.mm. Hypochromia and poikilocytosis were noted in a stained film. He was placed on an ordinary hospital diet supplemented by 3 mg. of crystalline aneurin by mouth daily. On August 21, 1939, a fractional test meal showed an appreciable increase in the amount of free HCl as compared with that present a few days after his admission. Clinically, the hyperaesthesia diminished, as well as the sensory loss which on September 2, the day on which he was evacuated from the Hospital, was present only in fingers and feet. Table showing effect of treatment:

Date.	Aneurin treatment.	Excretion of aneurin in micrograms.		
		24-hours.	3-hours (no B <sub>1</sub> ).	3-hours (+B <sub>1</sub> ).
30-31.7.37	3 mg. daily by mouth beginning 1.8.39	20	1	0
26-27.8.39		450	75	100

The results in the first of these cases, which have also been confirmed in other cases, suggest that large doses of aneurin by injection, which have been advocated by many in polyneuritis from avitaminosis, are unnecessary, since much of the substance is excreted in the urine. The second case suggests, furthermore, that in the average case, aneurin when given by mouth leads to a rapid body saturation, as judged by the responses to a test dose. Finally, experience has shown that despite a rapid saturation with aneurin, the clinical signs clear up exceedingly slowly.

The following case of mental confusion in a chronic alcoholic patient shows that not only may clinical improvement occur on a diet poor in vitamin B<sub>1</sub>, but the body storage of this vitamin may rise:

L. M. O., female, aged 48 years, was admitted to St. Francis Hospital on June 14, 1938. We are indebted to Dr. F. Butler for his notes on the case. For some years this woman had been employed as a barmaid, and had consumed moderate amounts of alcohol. In 1933 she gave up this work. Shortly afterwards she became depressed and began to take a red wine known in her locality as 'Vin Sec', of which she consumed 1 to 4 pints daily. She soon lost her appetite, neglected to take any breakfast, and seldom ate any solid food until the evening meal, which she shared with her husband; latterly, according to him, she had only 'picked at it'.

On admission she had to be placed in a padded cell owing to her acutely confused state. On the 16th she was more co-operative, but confused, disorientated, and partially amnesic. She showed well-marked nystagmus, slight loss of power on dorsiflexion of the feet, absent deep reflexes in all



limbs, and blunting to pin-prick and cotton-wool on the peripheral parts of the limbs, but muscular tenderness was absent. The heart sounds were 'tic-tac', pulse regular and 100 per minute, blood-pressure 120/75. There was a massive pitting oedema up to the middle of the thighs and over the sacrum. A mild degree of glossitis was present. The liver edge was just palpable. The urine contained a trace of albumen. On the following day she was confabulating freely and showed a typical Korsakow's syndrome. She was seen by one of us on June 22. The confusional symptoms had largely cleared up. She no longer confabulated, but was poorly orientated in time. The signs of a chronic polyneuritis were confirmed, and oedema was still very marked up to the middle of the thighs. The heart did not seem enlarged; the heart sounds were 'tic-tac'. She was transferred to the Courtauld Ward of the Middlesex Hospital on June 24. On that evening she appeared normal mentally, except for marked impairment of memory for recent events, but she was no longer disorientated. No oedema at all was then apparent on the lower limbs, although it had been well marked 48 hours previously. Dr. D. E. Bedford considered that the oedema was not of cardiac origin and that clinically he did not find any evidence of the so-called beri-beri heart. Inquiry into her diet before her illness showed the following result:

Protein gm.	Fat gm.	Carbohydrate gm.	Wine 2 pts.	Calories.	Aneurin.	A/NFC.
127	39½	127	(= 600 cal. approx.)	2,009	358.5	0.21

This diet is not only low in calories but, of more importance, the A/NFC ratio is below normal.

On admission to St. Francis Hospital this patient had been put on a special diet high in carbohydrate (2,000 cal.) and low in aneurin (500 micrograms). No other treatment, except sedatives, was given. This diet was continued until June 25, the day after her admission to the Middlesex Hospital. The rapid improvement in the state of mental confusion and the disappearance of well-marked oedema occurred during the time she was on a diet containing half the recognized minimum amount of aneurin to maintain health (1,000 to 2,000 micrograms). The oedema might be explained by a reduction in the plasma proteins, but an estimation carried out on June 26 showed the following figures—albumin 3.39 gm. per 100 c.c., globulin 2.27 gm. per 100 c.c., fibrinogen 0.50 gm. per 100 c.c. The slight lowering of the albumen figure does not suggest that this was the sole reason for the oedema.

Table showing effect of a low aneurin diet on excretion in a case of chronic polyneuritis with confusion:

Date.	Aneurin treatment.	Excretion of aneurin in micrograms.		
		24-hours.	3-hours (no B <sub>1</sub> ).	3-hours (+B <sub>1</sub> ).
21-22.6.39		16	—	—
25-26.6.39		59	11	8
	5 mg. begun orally on 26.6.39			
	10 mg. orally 7-14.6.39			
16-17.7.39		120	—	104

The twenty-four hourly specimen collected on June 22 showed a low figure. By June 26 the twenty-four hourly specimen contained an amount of aneurin much nearer the normal figure, although a normal diet was not given until the specimen had been collected. The excretion after the test dose was, however, very low. The high figures obtained on July 17 can be accounted

for by the fact that from July 7 to July 14 she had 10 mg. of aneurin by the mouth daily. The rise in the amount of excreted aneurin which occurred when she was given a diet low in aneurin suggests that her previous reserves of this substance must have been very low, so that a diet containing the small amount of 500 micrograms appreciably increased the amount of this substance in the urine. In mental institutions it has long been recognized that certain types of mental confusion improve rapidly if adequate nourishment is given. One must be guarded, however, from drawing conclusions as to the part played by deficiency in aneurin in this and similar cases. Spies, Cooper, and Blankenhorn (1938) have recently reported their results of treatment with nicotinic acid of 60 cases of acute psychosis associated with pellagra. For several days some of the patients were put on a much restricted diet consisting of 100 to 200 gm. of glucose with unlimited water. If no improvement occurred in the mental condition, 500 to 1,000 mg. of nicotinic acid were given by the mouth. They reported prompt improvement in the mental picture following this therapy. It is possible that both aneurin and nicotinic acid deficiency may play a part in the acute confusional states that may occur in avitaminotic patients.

#### Summary

1. A modified thiochrome method for the detection of aneurin has been evolved. We consider that this method is sufficiently accurate for clinical purposes, and has an advantage over other methods for the detection of aneurin by its relative simplicity.

2. The amount of aneurin excreted after a test dose of 1 mg. given by the mouth is a more reliable guide than a twenty-four hourly estimate.

3. This test is of value in cases of polyneuritis of uncertain origin, in which an accurate dietary history has been difficult to obtain. The test can be used as a guide to treatment.

4. Our limited experience suggests that it is unnecessary to give aneurin subcutaneously, unless vomiting or marked diarrhoea is present. A daily dose of 3 mg. by mouth leads in the average case to a rapid saturation.

We wish to express our thanks to Miss V. Scott Carmichael who worked out the dietetic histories, and to Professor E. C. Dodds for helpful criticism. This work was done during the tenure of a Mackenzie Mackinnon Research Fellowship of the Royal Colleges of Physicians and Surgeons by Mr. G. M. Hills.

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## WERNICKE'S ENCEPHALOPATHY: THE CLINICAL FEATURES AND THEIR PROBABLE RELATIONSHIP TO VITAMIN B DEFICIENCY<sup>1</sup>

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SINCE Wernicke in 1881 described what he called acute superior haemorrhagic polio-encephalitis, the pathology of the condition has been described in several exhaustive papers, mainly in the German literature. Traditionally associated with alcoholism, its not infrequent occurrence as a complication of other conditions in non-alcoholics has been recently recognized (Neubürger, 1936, 1937; Campbell and Biggart, 1939).

We shall not here discuss the pathological features in any detail. They are summarized by Campbell and Biggart (1939), and references to the chief descriptions of the pathology are cited in that paper. Briefly, the lesions consist of foci of vascular stasis and parenchymatous degeneration, occurring symmetrically in the corpora mamillaria, and also, though not constantly, in other parts of the hypothalamus, the fornix, the juxtaventricular zone of the thalamus, the peri-aqueductal grey matter of the midbrain, the posterior colliculi, and the floor of the fourth ventricle. Lesions are occasionally found also in other areas, such as the corpus striatum, the substantia nigra, the anterior colliculi, the optic nerves, and, rarely, in the cerebral cortex (see Table III).

The 12 cases on which the pathological study referred to was based, together with nine further cases since observed, have convinced us that Wernicke's encephalopathy is a not uncommon condition, that it occurs as a complication of a wide range of other diseases, that its incidence in some of these diseases is not generally recognized, and that its symptoms and signs often form a syndrome which makes it possible to diagnose the condition during life. It seems desirable, therefore, to describe the clinical features on the basis of our series of 21 cases, which are separated in detail in the Appendix. As Wernicke's disease often occurs as a terminal complication of other diseases, complete clinical records have not always been available, but all our cases have been confirmed by autopsy.

Table I shows the conditions with which Wernicke's disease was associated. It can be seen that in nearly all our cases the primary disease was one

<sup>1</sup> Received September 15, 1940.

characterized by chronic gastro-intestinal disorder. The traditional cause, alcoholism, with its grossly reduced food intake, obviously falls into this category, but it accounts only certainly for five, and possibly for eight cases. We attribute the high proportion of non-alcoholic cases to the fact that we have carefully searched for the Wernicke lesions at autopsy in a large number of cases of fatal gastro-intestinal disease, especially when there was any history of disturbance of memory or consciousness before death. We were stimulated to do so by Neubürger's (1936, 1937) reports on the occurrence of Wernicke's disease in cases of gastric carcinoma and chronic gastritis. Neubürger was the first to widen the outlook on the disease by stressing its non-alcoholic incidence, and emphasized the ease with which it could be overlooked both clinically and pathologically.

The association of Wernicke's disease with alimentary disturbance and with polyneuritis strongly suggests that a vitamin deficiency is concerned in its production. This is discussed below under 'Aetiology'.

### *Clinical Features*

The duration of the encephalopathy varies considerably, in our series, from ten weeks to one day. There is no significant difference in duration between the alcoholic and the non-alcoholic cases, and in each group cases may be either acute or subacute (see Table I). For the whole series the average duration was 17 days, omitting cases 5 and 11, in which there were no symptoms of Wernicke's disease, and the pathological lesions appeared to be inactive or healing. The clinical phenomena fall into two groups, (1) disturbances of consciousness and of higher cerebral functions; and (2) focal neurological signs and symptoms.

*Disturbances of consciousness and of higher cerebral functions.* Such disturbances were a constant feature of our cases. In some patients drowsiness passing into coma dominated the picture; in others drowsiness and apathy alternated with periods of excitement and delirium. In such cases more specific psychotic symptoms could not be observed. In other patients, however, where drowsiness or delirium was slight or absent, symptoms of the Korsakow type were seen, disorientation, amnesia, hallucinations, and confabulation; occasionally a patient remained fairly alert while showing these symptoms. Case no. 6, for instance, described his hallucinations with considerable insight, saying that he 'saw' his friends at the foot of his bed as if he were 'dreaming without being really asleep'.

Kant (1932) believes that in chronic alcoholism there is a constant association between Wernicke's encephalopathy and Korsakow's psychosis; he has never seen a case of fatal Korsakow's psychosis without Wernicke lesions, and he believes that the typical Korsakow psychosis always appears in Wernicke's encephalopathy, provided the patient lives long enough and the degree of drowsiness or delirium is not sufficient to mask the characteristic features of the psychosis. Our material supports this view in that we also have never seen a case of fatal Korsakow psychosis, alcoholic or non-alcoholic,

which did not show Wernicke lesions; many of our cases of Wernicke's encephalopathy did not show an obvious Korsakow psychosis, but that might reasonably be explained by the masking effect of drowsiness or delirium, or the exhaustion produced by the primary disease. Carmichael and Stern (1931), however, did not find Wernicke lesions in a series of five fatal cases of Korsakow's psychosis.

TABLE I

*Twenty-one Cases of Wernicke's Encephalopathy*

Case No.	Sex.	Age.	Associated disease.	Duration of cerebral symptoms.	Presence and duration of polyneuritis.
1.	F.	45	Alcoholism	10 days	4 weeks
2.	M.	57	Alcoholism	4 weeks	
3.	F.	42	Alcoholism	1 week	6 weeks
4.	M.	37	Alcoholism	9 days	1 day
5.	F.	52	Alcoholism, acute pancreatic necrosis, healed Wernicke lesions	—	?3 years
6.	M.	64	Gastric carcinoma, ?alcoholism	4 weeks	
7.	F.	48	Gastric carcinoma, tuberculous peritonitis	2 weeks	
8.	F.	66	Gastric carcinoma	5 days	
9.	M.	68	Gastric carcinoma	1 week	1 week
10.	M.	68	Gastric carcinoma	5 days	
11.	F.	51	Pyloric stenosis, post-operative cerebral thrombosis, healed Wernicke lesions	—	?6 months
12.	F.	61	Chronic dyspepsia, gall stones	10 weeks	10 weeks
13.	F.	22	Resection of bowel (for tuberculosis), macrocytic anaemia	several days	
14.	F.	18	Chronic septic peritonitis, intestinal obstruction, ileo-transverse colostomy	4 weeks	
15.	F.	22	Hyperemesis gravidarum	1 day	
16.	F.	24	Pregnancy with vomiting	?2 months	
17.	F.	56	Pernicious anaemia, subacute combined degeneration of the cord	7 days	some months
18.	M.	68	Bronchiectasis, moderate drinker	3 days	?2 years
19.	F.	23	Chronic pyosalpinx, urinary infection	4 weeks	
20.	F.	42	Meningovascular syphilis, ?alcoholism	12 days	
21.	M.	3½	?Whooping cough	3 days	

None of our cases, alcoholic or non-alcoholic, showed the clinical picture of delirium tremens. This is in agreement with Kant (1932), and in disagreement with Neubürger (1931), who believes that Wernicke lesions may produce this syndrome.

*Epileptiform convulsions.* This feature occurred only once in our series, in case no. 4. We cannot recall having seen it recorded previously in Wernicke's disease, so that it is obviously an atypical and unusual sign. It seems significant that case no. 4 was one of the only two cases in our series in which cerebral cortical lesions were found, consisting in both cases of widely disseminated foci of vascular disturbance and parenchymatous degeneration similar to the lesions in the typical sites. Cortical lesions, both in our own experience and from examination of the literature, are rare in Wernicke's disease, apart

from somewhat equivocal mild diffuse degenerative changes which have been described, but on the significance of which there is no agreement. So it seems justifiable to ascribe the epileptiform convulsions in case no. 4 to the cortical lesions. In our other case (no. 2) showing cortical lesions, no symptoms or signs were noted that we could attribute to these lesions. It may be suggested that in these two cases the cortical lesions had a different, coincidental, pathological basis. They had, however, the typical histological appearance of Wernicke lesions; and in case no. 4 at least the relatively young age of the patient, 37 years, and the absence of arterial disease eliminated arteriosclerosis, the only other likely cause of such lesions.

*Impairment of vision.* Impairment of visual acuity was reported in three of our cases, in one of which bilateral central scotoma and papilloedema were present. In a fourth case papilloedema was noted, but the patient's lethargy made it impossible to assess visual acuity. In two of the three cases with loss of vision, focal degenerative lesions were demonstrated at autopsy in the optic nerves. In our series optic nerve lesions were thus obviously a significant part of the Wernicke syndrome. The only previous identification of such lesions in Wernicke's disease which we have found is by Tanaka (1934), who reported the occurrence of axial optic neuritis in Human Milk Intoxication, a disease occurring in Japanese breast-fed infants which appears to have Wernicke's encephalopathy as its pathological basis.

Sheehan (1939), in a recent review of the pathology of hyperemesis gravidarum, mentions partial or complete blindness as occurring in the complicating cerebral syndrome; and it seems probable that in some of his cases this symptom was due, as in ours, to optic nerve lesions. In at least one of his cases which showed a typical Wernicke's encephalopathy at autopsy, there were retinal haemorrhages, and these may be an alternative pathological basis for the dimness of vision. Retinal haemorrhages were not seen in any of our cases, but they have been recorded by several authors (Albeck, 1922; Berkwitz and Lufkin, 1932; Stander, 1932; Tillman, 1934; Randall and Wagener, 1937; Waterman, 1939; Sheehan, 1939) in hyperemesis gravidarum and polyneuritis gravidarum. Cases cited by Tillman and by Randall and Wagener showed lesions at autopsy extremely suggestive of Wernicke's disease, although this diagnosis was not made; and Sheehan, as we have noted above, recorded retinal haemorrhages in a case which he identified as one of Wernicke's disease. It is tempting, therefore, to include them as a part of the picture of the encephalopathy, but it is striking that they have not, as far as we know, been found in Wernicke's disease complicating conditions other than hyperemesis. Until their wider incidence is demonstrated one must hesitate to include them in the pathogenetic entity of the encephalopathy.

*Oculomotor disturbances.* These constitute the most frequent and most important group of focal neurological signs and symptoms, and were present in at least ten of our cases. Inequality and irregularity of the pupils with loss of the reaction to light may suggest neurosyphilis; in case 12 typical Argyll Robertson pupils were present. Paralysis of the conjugate eye



movements was a striking feature of three cases. Diplopia and strabismus were also frequent, being noted in six cases. Nystagmus was noted in five cases, and was probably overlooked in others.

*Respiratory paralysis.* Disturbances of respiration of apparently central origin have previously been described in Wernicke's disease. Such disturbance was noted in only one of our series (patient no. 13, who showed progressive respiratory paralysis).

*Pyramidal tract involvement.* None of our cases showed loss of voluntary motor power which could not better be explained by peripheral neuritis (or subacute combined degeneration in case 17). A bilateral extensor plantar response was elicited only in one case (no. 19), apart from the case (no. 17) of subacute combined degeneration of the cord.

*Polyneuritis.* In nine cases there was clinical evidence of polyneuritis (associated in one case with subacute combined degeneration of the cord and pernicious anaemia). The polyneuritis was histologically confirmed in three cases.

*Cerebrospinal fluid.* The cerebrospinal fluid was examined in detail in seven cases (see Table II). Of these, one (no. 20) had meningovascular syphilis in addition to Wernicke's disease and showed corresponding changes in the cerebrospinal fluid. In the remaining six, the fluid was in each case clear and colourless. The cell count varied from 1 to 10 cells per c.mm. The Wassermann reaction was negative in all. The colloidal gold curve was within normal limits, the maximum abnormality being 000012200000. The total protein varied from 20 to 160 mg. per 100 c.c., the average of the six specimens being 71 mg. per 100 c.c. (in three of the six the figure was below 40 mg. per 100 c.c.). Globulin tests were slightly positive in two cases. The sugar content (three cases) was 63, 83, and 100 mg. per 100 c.c. The sodium chloride content was 733 and 720 mg. per 100 c.c. in the two cases examined.

TABLE II

*Cerebrospinal Fluid in Wernicke's Encephalopathy*

Case No.	Cells per c.mm.	Protein mg. %.	Globulin test.	Sugar mg. %.	Sodium chloride mg.	Wassermann reaction.	Colloidal gold test.
2.	none	25		68	733	negative	000000000000
4.	10	160		83	720	negative	
9.	1	40	negative			negative	0000000000
12.	6	60	slightly positive			negative	000012200000
16.	120	120	positive			slightly positive	
19.	20	20		100		negative	0000000000
20.*	200	200		76	652	slightly positive	4554210000

\* Meningovascular syphilis also present.

It is therefore evident that the cerebrospinal fluid in these six cases showed some increase of total protein or globulin in three cases, but no other abnormality of diagnostic value.

*Correlation of Clinical and Pathological Abnormalities*

There can be little doubt that the disturbances of vision, of the eye movements, and of respiration are due to the lesions in the optic nerves, periaqueductal grey matter, and the vagal nuclei respectively, but the localization of the lesions which cause mental confusion and the sleep disturbance cannot be determined with so much confidence. The correlation of the clinical and pathological features of each of our cases is not reliable, because serial sections of the brain-stem, which would be required for such a study, were not made. It is well known that lesions of the hypothalamic region may produce sleep disturbances. The effect exerted by lesions of the hypothalamus in disturbing consciousness and in causing mental disturbances, including hallucinations, provides a subject for discussion which need not be considered in detail. It is interesting to note that Lhermitte (1932) considers that posterior hypothalamic lesions may produce hallucinations. The apparent fact that lesions situated as in Wernicke's disease can disorganize mental functions, which are presumably subserved by the cerebral cortex and its underlying white matter, is a subject of great interest regarding which one can only speculate.

*Diagnosis*

A history of gastro-intestinal disturbances such as might produce deficiency disease was present in nearly all our cases, and in most of the cases reported in the literature. It is obvious that the appearance of mental disturbance in such a case should raise the possibility of the development of Wernicke's disease, particularly if a polyneuritis is already present. The mental disturbance is of variable type; sometimes it is relatively non-specific, consisting of drowsiness, delirium, or coma; frequently, however, it crystallizes into a Korsakow psychosis. Our records suggest that in cases of Wernicke's disease, in addition to mental disturbances, careful neurological examination will usually elicit some evidence of disturbance of the brain-stem, particularly the midbrain. Paralysis of conjugate eye movements is highly characteristic, but minor disturbances such as nystagmus, inequality of the size of pupils or their reaction, or disturbances of respiratory rhythm may also be of diagnostic value. When the mental changes alone are detected, and do not constitute a definite Korsakow psychosis, or when only the visual disturbances are present, the diagnosis may remain uncertain. As regards the mental changes, as Környey (1937) emphasizes, not every case of coma in a patient with carcinoma of the stomach is due to Wernicke's encephalopathy. Cerebral metastases and uraemia brought on by constant vomiting (Neubürger, 1937) are among the alternative causes, but when there is a combination of presumable nutritional deficiency, mental disturbance of the Korsakow type, and signs of a midbrain lesion Wernicke's disease may be confidently diagnosed.

*Recovery from Wernicke's Encephalopathy*

Although in the great majority of our cases the Wernicke's encephalopathy appeared to be either the chief cause of death or at least an impor-

tant factor in hastening death, two cases (nos. 5 and 11) in which death was due to unrelated 'accidental' causes (acute pancreatic necrosis and internal carotid artery thrombosis), showed old healed or healing Wernicke lesions, unaccompanied by any symptoms of the clinical Wernicke syndrome. In one of the cases the sole lesions found were an atrophy and gliosis of one corpus mamillare and a small focus of degeneration in the corresponding column of the fornix. That the lesions in these cases were true Wernicke lesions seems almost certain, because of their specific localization and because both cases showed one of the primary conditions likely to be associated with Wernicke's disease, chronic alcoholism and pyloric stenosis. Neubürger (1936) has also noted the occasional occurrence of old scars in the corpora mamillaria in chronic alcoholics and in cases of gastric carcinoma, and draws the conclusion, which our two cases support, that Wernicke's encephalopathy may be recovered from, or indeed may be abortive and unaccompanied by recognizable symptoms. Jolliffe, Bowman, Rosenblum, and Fein (1940) report striking cures produced with nicotinic acid in a related if not identical syndrome, while Professor D. M. Dunlop has recently observed the dramatic recovery of a case treated with vitamin B<sub>1</sub>. We are indebted to Professor Dunlop for the following notes on this case.

The patient, a married woman aged 57 years, was admitted to the Edinburgh Royal Infirmary on 11.5.40, complaining of vomiting and pains in her legs. Her illness had begun in February 1940, when she became easily tired, lost appetite, and developed swelling of her ankles. This swelling subsequently spread almost all over her body. In April 1940 she began to vomit, and this became more and more frequent, eventually occurring after every meal. Her calves then became numb and tender, her legs weak, and she grew very listless and drowsy.

On examination (11.5.40) she was pale, and there was great oedema of her legs and lower trunk. Her pulse-rate was 110 per minute, and her blood-pressure 140/80; the apex beat was in the sixth intercostal space, just outside the mid-clavicular line, and X-rays showed an enlarged, globular heart. The liver was slightly enlarged. The urine contained a little albumen and some granular casts. The temperature was normal. Her mental state was one of great drowsiness, apathy, and confusion, and her memory was very poor, but she was not obviously hallucinated. There was ptosis of the left eyelid, paralysis of both lateral rectus muscles, and paralysis of conjugate movement to the right; the pupil reactions and the other cranial nerve functions appeared normal. The retinae appeared healthy ophthalmoscopically. There was marked weakness of all leg muscles, the knee and ankle jerks were absent, and the biceps and triceps jerks were weak; the plantar responses were flexor; there was no tremor or rigidity. The calves were tender on pressure, but cutaneous sensation could not be tested owing to her drowsiness and apathy. The cerebrospinal fluid showed one cell per c.mm., protein 25 mg. per 100 c.c., globulin test negative, sugar 90 mg. per 100 c.c., sodium chloride 640 mg. per 100 c.c.

The accounts she gave of her diet were conflicting and unreliable, but it appeared to have been generally very inadequate before admission. She remained in the same state until 18.5.40, when vitamin B<sub>1</sub> therapy was

begun (Benerva Forte 10 mg. daily intramuscularly for nine days). The results were dramatic. Diuresis appeared at once, the daily urine output rising within twenty-four hours from 600 c.c. to 1,800 c.c. and in forty-eight hours to 2,500 c.c., and she rapidly lost weight as the oedema disappeared. The change in mental and nervous symptoms was also striking; within forty-eight hours of starting B<sub>1</sub> therapy the apathy and drowsiness had greatly improved, and in a few days her mental condition appeared normal. The ptosis and ocular palsies also cleared up within a few days, and the knee and ankle jerks returned. After the course of B<sub>1</sub> therapy and the disappearance of the oedema, the pains in the legs became more severe, and a course of nicotinic acid was given from 4.6.40 to 8.6.40, during which time the leg pains were considerably relieved. She was discharged on 28.6.40 apparently completely recovered.

The combination of oedema and polyneuritis responding so dramatically to vitamin B<sub>1</sub> clearly indicates a diagnosis of beri-beri, and the associated oculomotor palsies and marked mental changes of a type similar to those in many of our cases confirmed by autopsy strongly suggest that the beri-beri was complicated by Wernicke's disease. It should be noted that the Wernicke symptoms also responded very rapidly to B<sub>1</sub> therapy.

Further, it is important to note that nuclear ocular palsies may occur and be recovered from in cases of alcoholism, as in the following case.

A man, aged 40 years, was seen by one of us in April 1938. During the previous winter he had suffered much from diarrhoea and had treated himself daily with large quantities of gin. A week before examination there was severe vomiting. Diplopia developed with internal strabismus, and slight unsteadiness in walking. The left pupil was larger than the right, the conjugate movement of both eyes was defective to right and left, and there was coarse nystagmus on looking laterally. Upward and downward movements of the eyes were full. The right optic disk was slightly oedematous. Further examination elicited slight unsteadiness in gait of the cerebellar type, but no other abnormality. The reflexes were normal. The cerebrospinal fluid pressure was 160 mm., there were two cells per c.mm., the total protein was 50 mg. per 100 c.c., the Noguchi test for globulin was negative, the gold curve was 001110000000. The Wassermann reaction was negative in both blood and cerebrospinal fluid.

Recovery occurred quickly, but a year later signs of alcoholic polyneuritis and great nervousness developed, and it was found that he had taken a bottle of gin daily for the previous eight months. There were then no ocular disturbances.

Looking back on this case it seems likely that the ocular palsies in 1938 were of the alcoholic type and were due to the central lesions of Wernicke's disease.

#### *Aetiology*

This has recently been discussed in some detail (Campbell and Biggart, 1939). For some time the theory held sway (Neubürger, 1936; and others) that the essential causal factor was a depression of the detoxicating action of the liver, which allowed endogenous toxins from the gut to reach the brain and so produce the encephalopathy. The evidence for this was never

convincing. It seems much more probable that the important factor is a deficient vitamin intake, most probably of some part or parts of the B complex. There is experimental evidence to support this, in particular the work of Alexander, Pijoan, Myerson, and Keane (1938) and Alexander (1940), who report the production of haemorrhagic lesions in the basal ganglia and brain-stem in pigeons by a diet deficient in vitamin B<sub>1</sub>, the lesions being strikingly similar to those of Wernicke's encephalopathy in man. In most of our cases, as in most of the cases of Wernicke's disease recorded in the literature, the probability, or at least possibility, of deficient vitamin intake or absorption is obvious from the nature of the primary condition of which the encephalopathy is a complication, and the frequency of an accompanying polyneuritis strongly incriminates vitamin B<sub>1</sub>.

Recently, however, Jolliffe, Bowman, Rosenblum, and Fein (1940) have reported a large series of cases of 'nicotinic acid deficiency encephalopathy'. This condition was characterized by 'clouding of consciousness, cog-wheel rigidities of the extremities, and uncontrollable grasping and sucking reflexes'. These authors state that it may occur as the only manifestation of deficiency, or with pellagral symptoms, B<sub>1</sub> polyneuritis, oculomotor disturbances of 'central neuritis', or scurvy. They give no autopsy findings, but their cases, which occurred mainly in chronic alcoholics, are in their opinion clinically identical with some of the cases described by Bender and Schilder (1933) as 'encephalopathia alcoholica', the pathological basis of which appears to have been typical Wernicke lesions. The clinical picture in the cases described by Jolliffe, Bowman, Rosenblum, and Fein is certainly very similar to that of Wernicke's encephalopathy as observed by us, except that we have not observed the cog-wheel rigidity and grasping and sucking reflexes that they describe. They state that vitamin B<sub>1</sub> therapy had no effect in their cases, while nicotinic acid had a striking and rapid therapeutic effect. They believe that the oculomotor disturbances when present 'are a manifestation of a disease process distinct from this specific encephalopathic syndrome'.

Symptoms of the pellagra syndrome were present in only half of the cases observed by Jolliffe, Bowman, Rosenblum, and Fein. They were observed in a much smaller proportion of our cases, although they may have been missed in some; glossitis was present in two cases (one with pernicious anaemia) and glossitis and stomatitis in one. In contrast, polyneuritis was probably present in nine of our cases. A deficiency of vitamin B<sub>1</sub> is therefore apparently more prominent than that of nicotinic acid. The therapeutic results reported by Jolliffe, Bowman, Rosenblum, and Fein (1940) are so striking that one must seriously consider nicotinic acid deficiency as a factor in the pathogenesis of Wernicke's encephalopathy. It may be that both vitamins are concerned. These authors appear to separate the midbrain lesions responsible for the oculomotor disturbances from the essential picture of their encephalopathy, presumably because their patients did not respond in the same way to nicotinic acid, although this is not stated.

It might be suggested that the midbrain lesions are due to vitamin B<sub>1</sub> deficiency, while the rest of the syndrome is due to nicotinic acid deficiency. However, the lesions in the corpora mamillaria and other parts of the hypothalamus are histologically of exactly the same type as those in the midbrain, and, incidentally, not at all of the same type as those found in the brain in pellagra proper, so that one feels averse from postulating two different pathogeneses for them. It is difficult, also, to believe that the hypothalamic lesions are not concerned in the production at least of the drowsiness found in so many cases of Wernicke's disease. From the pathological point of view it seems more probable, therefore, that the oculomotor disturbances and a part at least of the disturbances of consciousness form an entity. It may be that this entity (Wernicke's disease) is due to combined deficiency of vitamin B<sub>1</sub> and nicotinic acid, or it may be that on this entity there may be superimposed a further clinical syndrome due to nicotinic acid deficiency alone. In other words, Wernicke's disease may be combined with nicotinic acid deficiency encephalopathy, and that the latter may sometimes be the predominant partner, as in the cases quoted by Jolliffe, Bowman, Rosenblum, and Fein. Their cases differed from ours in the occurrence of cog-wheel rigidities and grasping and sucking reflexes. These phenomena may perhaps be the hall-mark of the nicotinic acid deficiency element, but in our experience they are not a characteristic feature of Wernicke's disease.

None of these cases in our series proper, which were all fatal, was adequately treated with either vitamin B<sub>1</sub> or nicotinic acid, but the additional case, which we have cited above, of what appeared to be Wernicke's encephalopathy complicating beri-beri, responded dramatically to vitamin B<sub>1</sub>, and supports us in our belief that B<sub>1</sub> deficiency plays a part in the pathogenesis of Wernicke's disease. The obvious practical conclusion is, that till the question is decided, cases of Wernicke's disease should be treated with, if necessary, both vitamin B<sub>1</sub> and nicotinic acid, preferably, of course, not simultaneously.

In a small group of our cases (nos. 18 to 21) in which the primary disease was an infective one, the existence or probable existence of deficient vitamin intake was not so obvious. In cases nos. 19 and 21 we could see no reason for such a deficiency, and these cases remain mysterious in their pathogenesis. In cases nos. 18 and 20, however, alcoholism and a generally inadequate diet seemed not improbable though the histories were inconclusive.

### *Summary*

1. Twenty-one cases of Wernicke's encephalopathy in which the diagnosis was confirmed at autopsy are reported, and their clinical features are discussed.

2. The encephalopathy occurred as a complication of chronic alcoholism, gastric carcinoma and other gastro-intestinal disorders, pernicious anaemia, vomiting in pregnancy, and a small group of heterogeneous infective diseases.

3. The possibility of recovery from Wernicke's encephalopathy is discussed. Pathological evidence of healing of the lesions is recorded, and two cases of probable recovery are described, one in response to vitamin B<sub>1</sub> therapy.

We wish to express our gratitude to those physicians, surgeons, obstetricians, and pathologists of the Royal Infirmary, the Municipal Hospitals, the Chalmers Hospital, and the Longmore Hospital, Edinburgh, for putting at our disposal their clinical and autopsy records.

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## APPENDIX

*Case Reports*

*Case 1.* The patient, a woman aged 45 years, was admitted to the Edinburgh Royal Infirmary on 11.9.25. She was reported to be a chronic alcoholic, and had been subject, during the previous four years, to delusions of persecution which were mainly referred to her neighbours. These delusions were most noticeable during menstruation and were aggravated by drink. Seven weeks before admission the patient complained of dizziness and nausea, and went to bed. For three weeks she vomited after almost every meal, but there was no complaint of pain. Four weeks before admission the legs became weak. She was unable to draw them up in bed and they were painful when touched. A week later there was loss of power in both hands, and a complaint of 'pins and needles' in the fingers. A week before admission she became drowsy and slept all day, but would waken up for a short period in the evening and shout out. At this time she had vivid hallucinations of people stabbing her. During the previous four days she had been unable to give a coherent reply to questions and there had been incontinence of urine and faeces.

On admission she was found to be semi-comatose, but occasionally shouted out in reply to questions. She refused to open her eyes, but was suffering from hallucinations. The left pupil was dilated and slightly irregular, and both pupils were inactive to light. There was some flattening of the right side of the face, but the cranial nerves were otherwise normal. The limbs were all flaccid, especially on the right side. The knee and ankle jerks were absent. There was loss of control of the sphincters. The muscles were sensitive to pressure. The cerebrospinal fluid was clear and not under pressure; films showed a few lymphocytes and there was no growth on culture. The pulse was 84 per minute, temperature 98° F., and the blood-pressure 110/75. The urine contained albumen. There was some bleeding from the vagina, but vaginal examination revealed no abnormality. There was an ischio-rectal abscess on the right side. The patient became progressively more comatose and died three days after admission.

Autopsy showed: Wernicke's encephalopathy, with subacute lesions in the corpora mamillaria and other parts of the hypothalamus, the juxta-ventricular zone of the thalamus, the periaqueductal grey matter, and the posterior colliculi (see Table III), dilatation of the right ventricle of the heart, and pulmonary congestion.

*Case 2.* The patient, a man aged 57 years, was admitted to the Eastern General Hospital on 29.3.39. He was a coal trimmer, and had had an injury to his shoulder in October 1938. About the beginning of March 1939 it was noticed that he was becoming confused and that he became unable to recognize his neighbours or to carry on conversation. Finally he became unable to keep himself clean or to feed himself. He drank port wine regularly in a quantity sufficient to produce frequent intoxication.

On admission he appeared thin and cyanosed. He was semi-conscious, his speech was confused, and there was Cheyne-Stokes breathing. Blood-pressure was 130/90; the apex of the heart was 1½ inches lateral to the mid-clavicular line and there was a blowing systolic murmur propagated towards the axilla. There was also an aortic systolic murmur propagated to the vessels of the neck. There was no oedema. Examination of the lungs



TABLE III  
*Distribution of Lesions in Brain in Wernicke's Encephalopathy*

Region.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Cerebrum :																					
Corpora mamillaria	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Other parts of hypothalamus	+	+		+	?	+	+				+	+	+	+	+	+	+	+	+	+	+
Fornix	+	+	+	+		+			+										+	+	+
Thalamus (juxta-ventricular zone)	+	+		+	+		+		+	+			+		+	+			+		
Habenular nuclei								+													
Corpus striatum (anterior part, including caudate nucleus and putamen)								+				+			+						
Substantia nigra								+							+						
Cerebral cortex	+			+																	
Midbrain :																					
Peri-aqueductal grey matter	+	+		+		+	+		+	+		+		+	+	+		+	+	+	+
Anterior colliculi						+	+			+								+	+	+	+
Posterior colliculi	+	+		+	+	+	+	+	+			+		+	+	+		+	+	+	+
Hind-brain :																					
Floor of fourth ventricle						+			+							+	+				
Cerebellar cortex								+	+												+
Optic nerves										+											

*Note:* This table cannot claim to be complete, and every region listed was not examined microscopically in every case.

revealed evidence of basal congestion on both sides. The pupils were small and unequal, the left being larger than the right; both reacted to light. There was nystagmus on looking to the left. The optic disks were normal. The tendon reflexes were present, with the exception of the ankle jerks, which were not elicited. The plantar response was flexor on both sides. The bladder was distended; the urine contained no albumen. The cerebrospinal fluid was clear and colourless, no cells were seen, the protein was 25 mg. per 100 c.c., the colloid gold curve was 000000000000, the Wassermann reaction was negative, the sugar was 68 mg. per 100 c.c., and the sodium chloride 733 mg. per 100 c.c. The blood Wassermann reaction and the Kahn test were positive. The patient slowly became weaker, but no further physical abnormalities appeared. Death occurred on 5.4.39.

Autopsy showed: Wernicke's encephalopathy (see Table III), dilatation of heart, chronic venous congestion of liver, and hypostatic pneumonia.

*Case 3.* The patient, a woman aged 42 years, was admitted to the Edinburgh Royal Infirmary on 26.3.39. Her husband said that following the death of her father-in-law in 1936 she had become depressed and began to drink to excess. She was frequently under the influence of whisky and injured herself with several falls. In November 1938 she fell in the fire and was severely burned. She had been more or less confined to bed since then, and during the last three months of her life had been unable to take food, but had continued to drink about a bottle of whisky a day. About five weeks before death she complained of difficulty in walking, and loss of feeling and swelling of the feet. This became gradually more marked, and a week before death she became mentally confused. She began to imagine that she had been put in terrifying dungeons and tunnels and these hallucinations were very vivid. She was afraid of being left alone and though she would sleep all day was unable to sleep at night, so that her husband had to stay up with her throughout the night. She then gradually became more confused and developed incontinence of urine. On admission she was completely disorientated for time and place and was unable to recognize relatives. She appeared to see people that were not there. Her memory of recent events was greatly impaired. There was gross oedema, weakness of the limbs with absent reflexes, and tenderness of the muscles. She gradually became more deeply unconscious, developed respiratory embarrassment and high fever, and died on 31.3.39.

Autopsy showed: Wernicke's encephalopathy (see Table III), polyneuritis, fatty degeneration of liver, bronchiolitis, old healed gastric ulcer, and gastrojejunostomy.

*Case 4.* The patient, a man aged 37 years, was admitted to the Edinburgh Royal Infirmary on 25.4.40. He had been drinking heavily for the previous year, recently a quart of whisky a day. Six months previously he had had an illness thought to be delirium tremens. On recovery from this he remained careless about his clothing, and would take little or no food, but no definitely psychotic symptoms were noted by his associates. On 20.4.40 he collapsed in a fit, after which he was confined to bed in a restless, irrational, incoherent state.

On admission he was ill nourished and semi-comatose, with temperature 99° F., pulse 130, respirations 22, and stertorous breathing. He appeared to understand what was said to him, but responded only by groaning. There was no sign of hemiplegia; the tendon jerks were all present and equal. There was no tremor or muscular rigidity. On 26.4.40 he had

a generalized epileptiform fit, with strabismus and nystagmus. Cerebrospinal fluid (27.4.40) showed: cells 10 per c.mm., protein 160 mg. per 100 c.c., sugar 83 mg. per 100 c.c., sodium chloride 720 mg. per 100 c.c., Wassermann reaction negative. Blood urea nitrogen was 12 mg. per 100 c.c. On 28.4.40 he showed foot and wrist drop. He alternated between restlessness and stupor till his death on 29.4.40.

Autopsy showed: Wernicke's encephalopathy (see Table III), polyneuritis, fatty degeneration of liver, and bronchopneumonia.

*Case 5.* The patient, a woman aged 52 years, died in the Edinburgh Royal Infirmary on 13.9.38, the cause of death being coronary thrombosis. She had been admitted to hospital two days previously suffering from severe cardiac symptoms and mental confusion. Her son reported that his mother had not been well for over 20 years. Every ten days or so she would retire to bed 'with severe headache and bile'. Her appetite was always poor, but her diet was varied. The sight of the left eye had always been defective. For many years she had been taking a bottle of cheap wine every day, but this never led to any acute physical or nervous breakdown. During the previous eight years he had noticed gradual deterioration in her memory, she had lost interest in her work at home, and neglected her duties in looking after the house. She remained rational, however, and was never in bed for more than a day or two at a time. During the previous three years she had complained of pains in the hands and feet and her walking had recently become unsteady. There had been numbness of the hands and feet for 18 months before death, but he had noticed no difficulty in her use of her hands. Her appetite had been even worse lately and her average diet consisted of a cup of tea for breakfast, one potato and a little meat or vegetable for lunch, one sandwich and a cup of tea for tea. There had been loss of weight during the previous six months and considerable breathlessness on exertion which had gradually become worse. There was no history of any illness associated with disorientation or disturbance of the eyes, nor was there any period in which the sleep rhythm was disturbed.

Autopsy showed: healing Wernicke's encephalopathy, coronary atheroma, and thrombosis with recent myocardial infarction. Lesions were found in the corpora mamillaria and the posterior colliculi. The corpora mamillaria showed macroscopically gross symmetrical atrophy, and microscopically gliosis without evidence of active degenerative changes. The lesions in the posterior colliculi were also relatively inactive, though compound granular corpuscles were still present in them, in contrast to the lesions of the corpora mamillaria.

*Case 6.* The patient, a man aged 64 years, was admitted to the Edinburgh Royal Infirmary on 12.1.39. He had first complained of loss of appetite in November 1938. He had taken alcohol regularly all his life and was accustomed to become intoxicated every week-end. This he had done to his doctor's knowledge for at least twenty years. His wife died in 1937 and his diet since then had not been so satisfactory, as he had had to look after himself. His food had consisted of tea and bread, with meat and fish occasionally. Three weeks before admission to hospital he developed epigastric pains which came on an hour after meals, and there had been vomiting for two weeks.

On examination the patient was emaciated and there was a palpable swelling in the epigastrium. A test meal showed that there was complete histamine-fast achlorhydria. X-ray examination and gastroscopy showed

a carcinoma involving the lesser curvature of the stomach. The blood-urea nitrogen was 21 mg. per 100 c.c.; the Wassermann reaction was negative. Operation was performed on 26.1.39 by Mr. Graham. It was found impossible to remove the tumour and gastro-enterostomy was carried out. While convalescent from the operation he developed hallucinations and thought he could see friends and relatives at his bed-side. When examined on 24.2.39 he appeared emaciated, but was quite alert and cheerful. He did not know the name of the hospital and had no memory of his operation. He was unable to name the day of the week, the month, or the year. He could read correctly and could write his name, but memory tests were defective. He was unable to repeat six digits and failed to subtract seven from a hundred repeatedly. He could name objects correctly. He described how he saw two or three of his friends at his bed-side. The vision was very vivid, but he realized that they were not really there. He sometimes had double vision. The optic disks were normal, visual acuity was J1 on the right and J4 on the left. The pupils were unequal, the left being slightly larger than the right, but they were normal in size; both reacted to direct and consensual light and to accommodation. There was a slight internal strabismus and slight impairment of the outward movement of both eyes; upward movement was completely absent and the downward conjugate movement of the eyes was grossly defective. There was irregular nystagmus of the oscillatory type with the eyes at rest; this was more marked on looking laterally and on looking to the left became phasic with the quick component to the left. The visual fields were full to rough tests. The reflexes were normal and there was no sensory loss even of vibration sense. There were no peripheral paraesthesiae and the muscles were not unduly sensitive. There was no marked anaemia. The blood-pressure was 130/95 and the pulse-rate was 85 per minute. Examination of the heart and lungs revealed no abnormality. The tongue was furred but moist, and there was some pyorrhoea. The patient gradually became weaker and died in Longmore Hospital on 2.3.39.

Autopsy showed: Wernicke's encephalopathy (see Table III), and carcinoma of stomach with gastrojejunostomy.

*Case 7.* The patient, a woman aged 48 years, was admitted to the Western General Hospital on 31.12.36. The history obtained from the relatives was that for four months she had been complaining of tiredness, loss of weight, and mental depression. About two weeks before death she became incoherent and her speech could not be understood. She did not appear to have hallucinations and there was no marked restlessness. When she tried to get up she staggered with weakness. She became progressively more confused and difficult to rouse. Two days before death she had a rigor and the temperature rose to 101.5° F. She was thought to have pneumonia and was sent to hospital. No paralysis or disturbance of vision was noticed. Her appetite had been very poor in recent months, but vomiting seldom occurred. One foot had been amputated for tuberculous disease, but no other illness was known, and there had been no previous mental disorder. She began to diet for obesity about two years before and her voluntary reduction of food merged into the loss of appetite of her final illness. She was a non-smoker and strict teetotaler.

On examination she was semi-comatose and was unable to co-operate. Speech occurred spontaneously in the form of muttered irrelevant remarks. The pupils were equal and reacted to light and accommodation. The upper

limbs were held in the position of flexion and the reflexes were not elicited. The reflexes in the lower limbs were present and normal. Respirations were erratic, possibly owing to continual inarticulate remarks. No other pulmonary abnormality was detected. The tongue was furred and there was no abdominal tenderness or rigidity. The temperature rose to 105° F. and the patient died next day.

Autopsy showed: Wernicke's encephalopathy (see Table III), gastric carcinoma, tuberculous cervical lymphadenitis, endometritis and peritonitis, and hyperplasia of bone marrow.

*Case 8.* The patient, a woman aged 66 years, suffered from sickness and vomiting for four years before her admission to the Eastern General Hospital. Two weeks before death she became jaundiced and four or five days before death developed drowsiness and incontinence. There was no free hydrochloric acid in the gastric contents. The temperature was raised before death. Her friends stated that the patient did not drink. She was said to have been 'highly strung', but had not altered in mind or character in recent years.

Autopsy showed: Wernicke's encephalopathy (see Table III), carcinoma of stomach, and hypostatic pneumonia.

*Case 9.* The patient, a man aged 68 years, was admitted to the Edinburgh Royal Infirmary on 20.7.38. He was a night watchman. He took beer and whisky at times, but never to excess. He had been well until nine months previously when he had begun to suffer from loss of appetite and flatulence. These symptoms gradually became worse and he lost a stone in weight. There had been occasional vomiting during the previous few weeks.

On admission he was quite well nourished. The pulse-rate was 82 per minute, there was no pyrexia, and the respiration rate was 22 per minute. The blood-pressure was 124/64. The urine was normal and the haemoglobin content of the blood was 90 per cent. Gastric analysis showed that there was no free HCl and that lactic acid was present. A few days after admission he complained of dimness of vision and double vision. He became mentally confused and was found to have lateral nystagmus; the pupillary reactions were normal; the visual acuity was so poor that he was unable to count fingers. The knee and ankle jerks were absent. There was loss of sensation to pin prick over both legs and incoordination of both legs and arms. The calf muscles were hyperalgesic. The cerebrospinal fluid was found to contain 1 cell per c.mm., the globulin tests were negative, total protein was 40 mg. per 100 c.c., the colloidal gold curve was 0000000000, the Wassermann reaction was negative, and the fluid was sterile. Mental confusion and confabulation continued. Fever developed and death occurred on 2.8.38.

Autopsy showed: Wernicke's encephalopathy (see Table III), polyneuritis, carcinoma of stomach, and dilatation of heart.

*Case 10.* The patient, a man aged 68 years, was admitted to the Edinburgh Royal Infirmary on 14.4.40. He had been complaining of upper abdominal pain, nausea, and vomiting for the previous two to three weeks. On 9.4.40 he became confused, disorientated for time and place (he thought himself at a Masonic meeting), and rather excited. His vision became dim. On 12.4.40 he became extremely drowsy.

On admission he was semi-conscious, breathing stertorously, and apparently quite blind. His pupils were small and reacted sluggishly to light. His

knee and ankle jerks were equal and feeble, his abdominal reflexes were absent, and there was a doubtful extensor plantar response on both sides. He died a few hours after admission, i.e. five days after the onset of cerebral symptoms. His alcoholic consumption was said to be moderate, 'a nip and a half pint' nightly. Up till two to three weeks previously his diet appeared to have been satisfactory.

Autopsy showed: Wernicke's encephalopathy (see Table III), as well as the customary sites, lesions were present in the trunk of each optic nerve; gastric carcinoma, and hypertensive cardiac hypertrophy.

*Case 11.* The patient, a woman aged 51 years, was admitted to the Edinburgh Royal Infirmary on 24.6.38. She had for many years suffered from symptoms suggestive of duodenal ulceration and 18 months previously there had been haematemesis. After this haemorrhage the symptoms were less severe for a year, but six months before admission they returned and were associated with frequent vomiting. She also complained of pains in the legs and giddiness, but there was no swelling of the ankles. The patient had seven children, all of whom were alive and well.

On examination she looked ill and had a yellowish tinge in her skin, the tongue was dry and furred, and the skin was inelastic. Examination of the abdomen revealed no abnormality, and the heart and lungs were normal. The knee and ankle jerks were absent and the plantar response was doubtful. No sensory loss was detected. X-ray examination revealed deformity of the duodenal cap and an atonic stomach containing a large quantity of fluid after fasting. The blood count showed: red cells 3,410,000 per c.mm., haemoglobin 65 per cent., and the blood-film revealed no abnormality. Examination of the gastric contents before and after a test meal showed no free HCl. An operation was performed on 19.7.38 by the late Sir David Wilkie. The stomach was found to be greatly dilated and hypertrophied owing to chronic duodenal ulcer with stenosis. A gastrojejunostomy was performed. The gall-bladder contained numerous large stones and these were removed. Three days after operation the patient suddenly collapsed, became aphasic, and developed twitchings of the legs. Death quickly occurred.

Her husband subsequently said that his wife never took alcohol. Her health deteriorated rapidly a few weeks before admission to hospital. She was never confused and there had been no complaint of visual disturbance. She had been living on milk, puddings, and fruit, but had vomited one hour after almost every meal during the previous six months. She had also been complaining of numbness of the hands and feet. There had been no previous illnesses of note. Her seven pregnancies had all been uneventful and in none of them had vomiting occurred. There was no indication of previous illness suggestive of Wernicke's disease.

Autopsy showed: recent thrombosis of the left internal carotid artery, healed Wernicke's encephalopathy (old lesion in one corpus mamillare), post-ulcerative pyloric stenosis, recent gastrojejunostomy, and hypostatic pneumonia.

*Case 12.* The patient, a woman aged 61 years, was admitted to the Chalmers Hospital on 27.9.37. In 1909 a gastro-enterostomy had been performed. She had had dermatitis ten years before and again in 1934. In 1930 there was an acute pain in the right loin diagnosed as renal colic and in 1937 there was an acute upper abdominal pain which was thought to be due to gall-stones. Since 1937 she had not been as vigorous or active as

formerly. Two months before admission she was found to be walking unsteadily. Some vomiting developed and she retired to bed. She gradually became confused mentally and lost interest in her surroundings. She repeated questions, and her memory failed. Seven weeks before admission she became weaker and had 'queer turns' in which there was great lassitude and peculiar movements of her hands. A few days later her condition improved. She was able to get up, but could not walk without assistance, and began to complain of numbness in, first, the right hand and right leg and, later, the left hand and left leg. Five weeks before admission the patient had another 'queer turn' in which she gasped for breath and rubbed her hands together. She was violently sick on several occasions and became progressively more weak and helpless.

On examination the patient was very weak and could not move without assistance. She took no interest in her surroundings and could answer questions only vaguely and after persuasion. Her memory was poor and she was disorientated for both time and place. The optic disks showed no definite abnormality. The pupils were small and equal, and there was no reaction to light, but they contracted on accommodation. There was slow horizontal nystagmus on looking to the right and left. The ocular movements were full in all directions. Examination of the other cranial nerves revealed no abnormality. There was pronounced weakness and wasting of all muscles, and the movements of both upper and lower limbs were ataxic. The tendon reflexes were all absent and the plantar responses were both indefinite. All forms of sensation were impaired in the arms and legs. The cerebrospinal fluid was not under increased pressure, it contained 6 cells per c.mm., the protein was 60 mg. per 100 c.c., Noguchi test slightly positive, the colloidal gold curve was 000012200000, and the Wassermann reaction was negative. A blood count showed: red cells 4,340,000 per c.mm., haemoglobin 87 per cent., and white cells 5,600 per c.mm. The tongue was dry, raw, and fissured. The blood-pressure was normal. The urine contained sugar on one occasion. The patient gradually became comatose, and restless movements of the limbs continued. The breath was foul-smelling, the respirations were jerky and consisted of rapid inspiration with a pause between each. There was slight irregular pyrexia. She died on 10.10.37.

Further inquiry confirmed that her home conditions were good and that up to the onset of her present illness the patient had taken a good normal diet, except that she avoided fried foods and potatoes. She was definitely not alcoholic.

Autopsy showed: Wernicke's encephalopathy (see Table III), gall-stones, old gastro-enterostomy, and terminal cystitis.

*Case 13.* The patient, a woman aged 22 years, was admitted to the Edinburgh Royal Infirmary on 31.7.37, complaining of vomiting which had occurred frequently during the previous two years. At the age of six a piece of tuberculous bone had been removed from the right forearm. Between the ages of 12 and 13 the patient was treated on several occasions in a sanatorium for abdominal tuberculosis. At the age of 17 the caecum, ascending colon, and one foot of the ileum were resected for tuberculous disease. The patient's health was fairly good between the ages of 18 and 20, but when aged 20 she began to complain of fatigue, dimness of vision, giddiness, and flashes of light before her eyes. She went to bed, vomiting started again, the tongue became inflamed, and the complexion yellow. She was again treated in the sanatorium where the condition was diagnosed as pernicious anaemia and was greatly

helped by injections of Campolon. When aged 21 she was again in bed for five months suffering from vomiting, fatigue, and diarrhoea. A blood count on 1.4.37 showed: red cells 2,000,000 per c.mm.; haemoglobin 60 per cent., colour index 1.5, white cells 4,000 per c.mm. There was poikilocytosis and the red cells were well filled; no nucleated red cells were seen.

During the previous three weeks the tongue had been very painful, the mouth swollen, and there had been a return of vomiting and weakness. After admission to hospital the patient complained of headache, but there was no papilloedema. The urine contained no abnormal constituents. The patient gradually developed respiratory paralysis, and half an hour before death the respirations were only six per minute.

Autopsy showed: Wernicke's encephalopathy (see Table III), hyperplasia of bone-marrow, and slight splenomegaly.

*Case 14.* The patient, a girl aged 18 years, was admitted to the Edinburgh Royal Infirmary suffering from acute appendicitis on 11.6.38. Operation was performed the same day and the appendix was found to have perforated. Considerable distension and vomiting followed the operation, but slow recovery occurred and the patient was discharged to the Astley Ainslie Institution on 15.7.38. She had to be readmitted three days later owing to persistent abdominal pains and vomiting. On 22.7.38 signs of acute obstruction with faecal vomiting developed and operation was carried out; many adhesions were encountered and ileo-transverse colostomy was performed. Thereafter the patient's health slowly deteriorated and it was necessary to give repeated doses of morphia and atropine to relieve the pain. She became very emaciated, apathetic, and difficult to deal with. Her speech became indistinct and her movements slow. There seemed to be facial contortions when she tried to speak. She relapsed into a state of muttering delirium for a month before death occurred on 21.9.38. When examined a week before death the pupils were both contracted and reacted very sluggishly to light, but morphia was being administered regularly. The inward movement of the left eye showed slight fatigue on sustained conjugate deviation to the right, but this was not observed two days later. Full examination of the nervous system revealed no further disturbance.

Autopsy showed: Wernicke's encephalopathy (see Table III), retrocaecal abscess, peritoneal adhesions, ileo-transverse colostomy, pyaemic abscesses in lungs, fatty degeneration of the liver, and cutaneous purpura.

*Case 15.* The patient, a woman aged 22 years, was admitted to the Maternity Hospital seven weeks before term. Six weeks previously she had become restless and depressed. Three weeks previously vomiting had commenced and became very severe during the week before admission, but stopped two days before admission. The patient became comatose and the doctor who was called in discovered the pregnancy, which was previously unknown to the parents.

On admission on 4.10.31 the patient was dazed and incoherent. There was slight cyanosis, but no jaundice or oedema. The blood-pressure was 150/115. The pulse was 120 per minute, respirations were 24 per minute, the temperature was 99.8°F. The tongue was very furred. The pupils were equal, regular, and reacted to light. There was no nystagmus. The knee jerks were present. There were no twitchings or spasms. Speech was indistinct. The urine contained albumen, but no sugar, acetone, or bile, and its specific gravity was 1010. Caesarian section was performed on 4.10.31 under spinal



anaesthesia, but after operation the patient became comatose and died the next day.

Autopsy showed: Wernicke's encephalopathy (see Table III), and fatty degeneration of the liver and kidneys.

*Case 16.* The patient, a woman aged 24 years, was admitted to the Edinburgh Royal Infirmary on 13.4.38. She was unmarried and about five months pregnant. Her appendix had been removed three months previously and at first she appeared to be having a normal convalescence, but for the last two months she had suffered from continual retching, progressive weakness, and loss of weight. There was increasing apathy with periods of drowsiness, but no other disturbance of the higher cerebral functions. Her own doctor had found neither albumen nor sugar in the urine, but, on admission, sugar and some acetone were found in the urine. The carbon dioxide combining power of the plasma was 63 vols. per 100 c.c.

On admission she made some spontaneous movements and the corneal reflex was present, but she could not be made to respond to questions or to instructions. Her pupils were small and did not react to light. Slight strabismus was noted. She was treated with insulin, glucose and sodium bicarbonate, and stimulants, but died next morning.

Autopsy showed: Wernicke's encephalopathy (see Table III), venous congestion of lungs, liver, spleen, and kidneys, and cloudy swelling of liver, kidneys, and heart.

*Case 17.* The patient, a woman aged 56 years, was admitted to the Edinburgh Royal Infirmary on 17.10.37. She was a paper-mill worker. Her sister said that she had suffered from swelling of the feet for two years, general weakness for six months, and vomiting for one week. Two years previously her feet not only became swollen, but she had difficulty in keeping her balance, and became very pale. She collapsed at her work a few weeks later and had not worked since. After spending six weeks in bed her condition improved, but the ankles still showed swelling. Six months before admission her walking became very unsteady and she was obliged to return to bed. A week before admission to hospital she began to vomit bile-stained fluid to such an extent that her doctor thought that intestinal obstruction might have developed. There was numbness of her fingers, hands, and legs. Her appetite was very poor, but there had been no gastric symptoms until a week before admission. There were no previous illnesses of note; the family history and social conditions were satisfactory. There was no question of her taking alcohol. She had been living during the past two years on a 'milk diet'.

On examination the patient was lethargic, the pupils reacted normally to light, the tendon reflexes were absent, and there was a bilateral extensor plantar response. There was loss of position sense in the legs and numbness of both fingers and legs. There was retention of urine, and a specimen showed no sugar, but abundant acetone. The blood-pressure was 118/82. There was no free HCl in the vomit. There was atrophic glossitis and a blood count showed red cells 3,500,000 per c.mm., haemoglobin 78 per cent., colour index 1.1. The carbon dioxide combining power of the plasma (18.10.37) was 63.5 vols. per 100 c.c. The cerebrospinal fluid on 20.10.37 was found to contain 1 cell per c.mm., the globulin test was positive, and the protein content was 120 mg. per 100 c.c. The Wassermann reaction was a doubtful positive. On 22.10.37 the patient was found to have papilloedema, bilateral partial third nerve paralysis, and some jerking movements of the jaw. She died on the same day.

Autopsy showed: Wernicke's encephalopathy (see Table III), subacute combined degeneration of the cord, hyperplasia of bone marrow, and siderosis of liver and spleen.

*Case 18.* The patient, a man aged 68 years, was admitted to the Edinburgh Royal Infirmary on 5.7.37. The history was that he had been unable to work for nine months owing to heart trouble. An internal strabismus had been noticed and he had been suffering from delusions.

On admission he was unconscious and unable to speak. The pulse was 130 per minute, the blood-pressure 104/68, and the apex beat was displaced to the left. No abnormality was noted on examination of the central nervous system. The temperature varied from 97.8° to 99.2° F. Death occurred next day. Subsequent inquiry from the patient's doctor elicited the information that two years previously he had suffered from a skin eruption on the front of both legs which was irritable and was associated with pain and numbness, and also some loss of power in both legs. This his doctor thought due to peripheral neuritis. His mental condition was not affected until two or three days before death, when he had developed disorientation and delusions. There was no vomiting. He was a moderate drinker.

Autopsy showed: Wernicke's encephalopathy (see Table III), bronchiectasis with multiple abscesses in the lower lobe of the left lung, fatty infiltration of the heart, dilatation of the right ventricle, and cloudy swelling of the heart and liver.

*Case 19.* The patient, a woman aged 23 years, was admitted to the Edinburgh Royal Infirmary on 20.5.38. She had always been backward mentally and had suffered from tuberculosis of the bowels in infancy and of the cervical glands four years previously.

On admission she complained of pain which had been present for six weeks in the left iliac fossa. There was some swelling of the abdomen and an offensive brown leucorrhoea. There was occasional vomiting, but the appetite was good. While in the ward she suffered from rigors and severe pyuria. Great improvement followed treatment with Prontosil and she was sent for convalescence to improve her condition for operation on what was thought to be an ovarian cyst. On admission to the convalescent hospital on 10.6.38 she seemed rather drowsy and vomited once or twice a day. These symptoms gradually became more marked and her appetite became poor. The haemoglobin was 60 per cent. Bilateral extensor plantar responses were reported. Vision became impaired, and she was found to have a central scotoma. The knee jerks were absent. On 28.6.38 mental disturbances were noted and she was readmitted to the Royal Infirmary on 2.7.38. She was then drowsy, but could be roused with difficulty; when awake she was childish and noisy, singing or whistling, and was confused and irritable. There was no evidence of hallucinations. Slight bilateral papilloedema was noted, but vision could not be satisfactorily tested. The conjugate eye movements were restricted in all directions except the downward movement which was full. There was slight left facial weakness. The tendon jerks were sluggish in the upper limbs, the knee jerks were absent, but the ankle jerks were present. There was a bilateral extensor plantar response. Examination of the cerebrospinal fluid showed: pressure 150 mm., total protein 20 mg. per 100 c.c., sugar 100 per 100 c.c., 1 lymphocyte per c.mm., colloidal gold curve

0000000000, and Wassermann reaction negative. The patient's condition deteriorated and she died on 12.7.38.

Autopsy showed: Wernicke's encephalopathy (see Table III), chronic left pyosalpinx, fibrinous peritonitis, acute splenic tumour, cloudy swelling of heart, liver, and kidneys, and pulmonary congestion and oedema.

*Case 20.* The patient, a married woman, aged 42 years, was admitted to the Edinburgh Royal Infirmary on 9.1.40. For the previous year she had complained of severe headaches. For the previous three months she had been emotionally unstable, had grown slovenly, her voice had become monotonous, and she had complained of attacks of dizziness. A week before admission she became very restless and excited, was confined to bed, her memory became much impaired, and she developed diplopia and a squint. She was thought to have hallucinations.

On admission the temperature, pulse, and respirations were normal. Her mental state varied between great drowsiness, muttering delirium, and periods of fair rationality; her memory was extremely bad. Her pupils were unequal, the right being dilated, and both were inactive to light and accommodation. There was a right sixth nerve paresis, causing diplopia and strabismus. Her tendon jerks were brisk and symmetrical; the plantar responses were flexor, and the abdominal reflexes absent. There was no loss of motor power. A blood count showed: haemoglobin 90 per cent. The Wassermann reaction was strongly positive. Cerebrospinal fluid, Wassermann reaction strongly positive, protein 200 mg. per 100 c.c., sugar 76 mg. per 100 c.c., sodium chloride 652 mg. per 100 c.c., and colloidal gold reaction 4554210000. Thereafter her temperature, pulse, and respirations rose steadily till death on 14.1.40, twelve days after the onset of acute cerebral symptoms. She was thought probably to have over-indulged in alcohol, and, in view of her apathy in recent months, to have had an unsatisfactory diet, but as she lived alone, no accurate information on these points was obtained.

Autopsy showed: meningovascular syphilis, Wernicke's encephalopathy, the lesions being of characteristic type, without evidence of syphilitic or other inflammatory phenomena, and bronchopneumonia.

*Case 21.* The patient, a boy aged 3½ years, was sent to hospital as a case of cerebrospinal meningitis on 26.12.36. The parents subsequently gave the history that the child was breast fed for three months and then given Nestlé's milk with orange juice. About the time of his illness there was no definite evidence of gross deficiency in his diet. There had been two other children, one stillborn owing to complicated labour. The patient was born five years later, and a year later the third child was born, but died aged 15 months. The parents were healthy. Two weeks before his illness he became listless and went off his food. His eyes watered freely, but were not inflamed. A week before his illness there was occasional vomiting just after food, with a slight cough. Three days before death the patient became feverish, very irritable, restless and sleepless, and appeared to have severe pain in the head. He seemed to be 'seeing things', saw a man on the ceiling and said 'take that man away'. The doctor thought the condition was due to meningitis and the patient was sent to hospital. No squint or other signs were noted.

On admission to hospital the patient was very irritable and there was head retraction with neck rigidity and a positive Kernig's sign. The tendon

reflexes were absent and the plantar response was flexor. The pupils reacted to light and there was no photophobia. The throat was congested, the tonsils enlarged, and the tongue heavily furred. The heart and lungs were normal and the skin was healthy. The cerebrospinal fluid was found to contain a few lymphocytes, but no polymorphs or organisms. Death occurred a few hours after admission. It was thought possible that he had been suffering from whooping-cough, but no evidence of this was seen during the brief period of observation in hospital.

Autopsy showed: Wernicke's encephalopathy (see Table III), no other significant lesions were found.

# THE VICIOUS CIRCLE IN CHRONIC BRIGHT'S DISEASE. EXPERIMENTAL EVIDENCE FROM THE HYPERTENSIVE RAT<sup>1</sup>

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With Plates 4 to 6

## *Introduction*

IN a previous communication (Wilson and Byrom, 1939) we described experiments in which persistent hypertension was produced in rats by partial occlusion of one renal artery, without interfering with the opposite kidney. Thirty-five rats were killed at short intervals after the onset of hypertension and the kidneys and other organs examined histologically. The opposite (unclamped) kidney showed lesions which bore a striking resemblance to those found in the human disease, 'malignant hypertension'. (Throughout this paper the term 'malignant hypertension' is restricted to the malignant form of essential hypertension.) These lesions included acute fibrinoid necrosis of the arterioles, endarteritis of the medium-sized arteries, acute changes in the glomeruli (including focal necrosis of the tuft, proliferative glomerulitis, capsular adhesions, and haemorrhagic infarction), and round cell infiltration in the interstitial tissue, changes which in the human kidney had been regarded as evidence of 'nephritis'. Such lesions were, however, absent from the clamped kidney. Other organs such as the heart, pancreas, and intestine showed acute necrosis of the arteries and arterioles similar to that occurring in these organs in malignant hypertension. The absence from the clamped kidney of lesions such as were present in the unclamped kidney was attributed to the protective action of the clamp in preventing the hypertension from reaching the renal arterial system distal to it, and this led to the conclusion that the pathological changes in the opposite kidney were a direct result of the increased blood-pressure.

If this conclusion is correct, it may help us to understand the pathogenesis of certain renal lesions in chronic hypertensive Bright's disease. Firstly, the occurrence in the unclamped kidney of the hypertensive rat of lesions closely resembling those found in malignant hypertension, provides experimental support for the view that the renal damage in this disease is the result and not the cause of the hypertension. During the past 30 years

<sup>1</sup> Received October 14, 1940.

this view has been held by certain clinicians and pathologists (Jores, 1916; Lohlein, 1917; Klemperer and Otani, 1931; Fishberg, 1931). In this country the influence of Allbutt's teaching, that renal involvement does not occur clinically in 'hyperpiesia', has prevented the recognition of a malignant form of essential hypertension. In recent years combined clinical and pathological studies of the disease in its various stages (Kimmelstiel and Wilson, 1936; Ellis, 1938) have shown that renal damage is minimal in the early stages, but develops rapidly as the disease progresses. On both clinical and experimental grounds therefore the renal sclerosis which characterizes this disease and led Fahr (1919) to give it the name 'malignant nephrosclerosis' is to be regarded as a result of the hypertension. Secondly, our experimental findings suggest the explanation of a further point which has given rise to much confusion; acute arteriolar necroses in the kidney, which are so characteristic of malignant hypertension, may also be found in other types of hypertensive Bright's disease, for example in chronic nephritis and occasionally in chronic pyelonephritis (Weiss and Parker, 1939). This difficulty disappears, however, when it is recognized that the lesions are specific, not for any one disease, but for the hypertension which is common to them all.

There is a still wider implication of these observations. Hypertension was produced in the rat by partial occlusion of a renal artery. It might therefore be expected that organic lesions of the renal arterial system in man would similarly give rise to hypertension. A vicious circle would thus be established in which hypertension led to acute occlusive vascular lesions in the kidney (arterial or glomerular) and these in turn caused a further rise in blood-pressure. The condition might then be expected to progress with ever-increasing rapidity, the terminal stages being marked by excessively high blood-pressure and severe renal destruction, with acute arterial lesions as a prominent feature. This would offer an explanation for the rapid downhill course which is so often seen in the final stages of any type of chronic hypertensive Bright's disease, and which we have termed the 'malignant termination'. If it can be substantiated, this conception of a 'vicious circle' would clear up much of the confusion which has arisen from the close similarity, both clinical and histological, shown by many cases in the terminal stages of various forms of chronic hypertensive Bright's disease. The present paper sets forth evidence in support of this conception derived from further experiments on the rat. In addition we shall describe a number of other observations bearing on the relation of hypertension to renal disease. The search for evidence of the existence of this vicious circle resolved itself into the following problems:

1. Do the acute lesions in the unclamped kidney described in our previous paper lead to permanent renal damage?
2. If so, are the character and extent of the damage such as might be expected to reduce the blood flow through the kidney, and thereby give rise to hypertension?

3. Do these chronic lesions resemble those of chronic hypertensive renal disease in man?

4. Does the high blood-pressure persist after removal of the clamped kidney? If the lesions in the unclamped kidney are such as might give rise to hypertension, we might expect this to be the case.

5. If such persistent hypertension occurs, can it be related to the degree of vascular damage in the unclamped kidney?

6. Can it be shown that this persistent hypertension is itself capable of causing further renal vascular and parenchymatous lesions?

### *Methods and Material*

Experimental hypertension was produced in 6 to 9 months old rats of both sexes by the silver clip method used on rabbits by Pickering and Prinzmetal (1938). Wistar, brown and white (Lister Institute), and cross-bred rats were used and all gave equally good results. The clips were made from silver ribbon (1.6 mm. broad and 0.13 mm. thick) which had been annealed by careful heating to a little below melting-point in a coal-gas flame. A strip 7.5 mm. long was bent into a U round the edge of a strip of copper foil 0.3 mm. thick, this thickness having been found suitable by repeated trial. Under ether anaesthesia the skin was shaved and the kidney exposed by a lumbar incision, gently freed from perirenal fat, and drawn outside the wound; it was next retracted forwards and held in position by a warm saline swab. In this way the renal vessels were exposed and slightly stretched; the renal artery and vein having been gently separated, the clip was hooked round the renal artery near its origin from the aorta and the ends of the clip closed with forceps. The kidney was replaced and the wound closed in two layers with cotton thread. Sepsis was a rare complication and in no case proved fatal.

The systolic blood-pressure was recorded weekly in all rats, and more frequently in some cases, under ether anaesthesia by the plethysmographic method of Byrom and Wilson (1938). Blood-urea estimations were made by a modification of the urease method on 0.2 c.c. of blood taken from the tail. The animals were killed, or died, at intervals ranging from 5 days to 46 weeks after constriction of the renal artery, and various organs were examined histologically after fixation in saline formaldehyde. Sections were stained by Ehrlich's haematoxylin and eosin, Weigert's fuchselin for elastic tissue, and Weigert's haematoxylin with Van Gieson's stain. Histological studies were made on 116 rats. At the outset sections were made from the majority of the organs. Later, only the kidneys, pancreas, mesentery, and heart were studied as these organs were found to be the sites of election for vascular lesions and we were primarily concerned with the renal changes. The eye and testis were examined only in selected cases. The frequency with which the various organs were examined is as follows: kidneys 114, heart 102, pancreas and mesentery 96, liver 31, intestine 29, stomach 17, suprarenals 20, testis 17, voluntary muscle 5, eye 7, spleen 2, brain, lungs, and bladder each 1.

*General Results*

Before passing to the specific problems of the investigation, some account is given of the course and duration of the hypertension in the series as a whole. The results of our experiments on renal artery constriction in rats differ in one important respect from the result of other workers using dogs or rabbits, namely that constriction of one renal artery in the rat, the other kidney being left intact, usually results in permanent elevation of the blood-pressure. We have performed this operation on 197 rats. Seventeen of these failed to develop hypertension, but are excluded from the series because they had been followed for less than three weeks and at that stage of the investigation it was not realized that hypertension may take longer than this to develop. Of the remaining 180 rats, 126 (70 per cent.) developed hypertension, and 54 (30 per cent.) failed to develop hypertension. Of the 116 animals in which histological studies were made, the blood-pressure was raised in 96 and normal in 20. The pre-operative blood-pressure varied from 78 to 135 mm., with a mean of 106 mm. These values are in close agreement with our previous figures for a series of normal rats. We have therefore regarded readings of 140 mm. or over (that is, nearly three times the standard deviation above the mean) as indicating hypertension (Byrom and Wilson, 1938).

The effects on the blood-pressure of constriction of one renal artery in the 126 rats which developed hypertension may be summarized as follows.

(a) A steep rise in blood-pressure (usually to 180 to 200 mm.) during the first week, often with severe constitutional disturbance, followed by death or by recovery with persistent hypertension.

(b) A gradual rise in blood-pressure over several weeks to a high level (180 to 230 mm.) which might be maintained for weeks or months. During this time constitutional disturbances, similar to those occurring at the onset in the first group, and described in detail below, frequently occurred.

(c) A gradual rise to a moderate level of hypertension (150 to 160 mm.) which was either transient or intermittent.

It is impossible to give the exact incidence of these types of response as many rats were killed soon after the development of hypertension, but in the later experiments where the technique was well established and the observations were carried on for longer periods, approximately 70 per cent. of the animals developed hypertension, and these were fairly equally divided between the three groups.

The constitutional disturbances mentioned above we have termed 'vascular crises'. In our earlier paper we described them as occurring at the onset of hypertension, but we had not then observed them during the course of a chronic persistent hypertension. In fact, they may occur for the first time many months after clamping of the renal artery. In mild cases the rat shows no marked change, but simply appears inactive and apathetic for a day or two. The symptoms in severe cases are conspicuous; the rat which previously



appeared healthy becomes inactive, crouches in a corner of the cage, loses appetite, and rapidly wastes, the fur becomes tousled, the skin cold, and the healthy pink colour of the feet and tail changes to a greyish-blue; the blood-pressure may still be elevated, but it is often found to be subnormal, although it may rise steeply during the next few days to a high level (Fig. 2, c). The condition of the animal may then rapidly deteriorate, death occurring in convulsions or coma within a few days, and the blood-pressure falling terminally to a low level; in other cases the symptoms and low blood-pressure persist for several weeks, after which death or recovery follows. The blood-urea during these crises is within normal limits (Wilson and Byrom, 1939). From the post-mortem appearances we have reason to believe that the syndrome is due to the production of widespread acute necrotic arterial lesions with perivascular tissue necrosis; in fatal cases such lesions are always found in the heart and mesentery, and gross signs of heart failure may be present. For this reason we have applied the term 'vascular crisis' to the syndrome. We believe that such crises are brought about in animals exhibiting the symptoms at the onset by sudden development of hypertension and in animals with chronic hypertension by a sudden exacerbation. Occasionally the blood-pressure records have revealed such an exacerbation, but more often the symptoms have appeared suddenly and our next reading has shown a fall in blood-pressure to a low level. In 16 rats presenting this syndrome the clamped kidney was removed, and in the majority this was followed by marked improvement. One rat which appeared moribund before the nephrectomy made a prompt recovery and subsequently had a successful pregnancy.

#### *Nature of the Histological Lesions in Rats with Long-standing Hypertension*

The first three problems outlined in the introduction were investigated by studying the renal lesions occurring in the unclamped kidney of rats with long-standing hypertension. In the experiments described in our previous paper the majority of animals were killed within a few weeks of the onset of hypertension. For this investigation 49 rats were killed after hypertension had persisted for periods of 8 to 46 weeks. In Fig. 1 and Table I are shown the maximum and average systolic blood-pressures and the duration of hypertension for each member of the series. The maximum pressures vary from 144 to 250 mm. of mercury and the average pressures from 127 to 200 mm. The large difference in some animals between mean and maximum pressures is due to periods of low blood-pressure caused by vascular crises as described above; for the same reason the general level of hypertension in these rats is often considerably higher than the mean value calculated for the whole experimental period.

*Histological findings in the unclamped kidney.* To the naked eye the unclamped kidney commonly shows compensatory hypertrophy and its surface often presents a well marked granular appearance. Microscopically obvious lesions were found in the majority of rats in the series, and differed

from the acute necrotic lesions previously described, being of a more chronic nature. Thus interstitial fibrosis and tubular atrophy and dilatation are more pronounced, and the changes in arterioles and glomeruli suggest organization and healing of the acute necrotic changes. In a number of instances, however, both acute and chronic lesions are present in the same section, indicating the progressive nature of the process. In the more severe cases there is manifest reduction in the number of glomeruli, and if these are counted it is evident that this is not simply an apparent reduction attributable

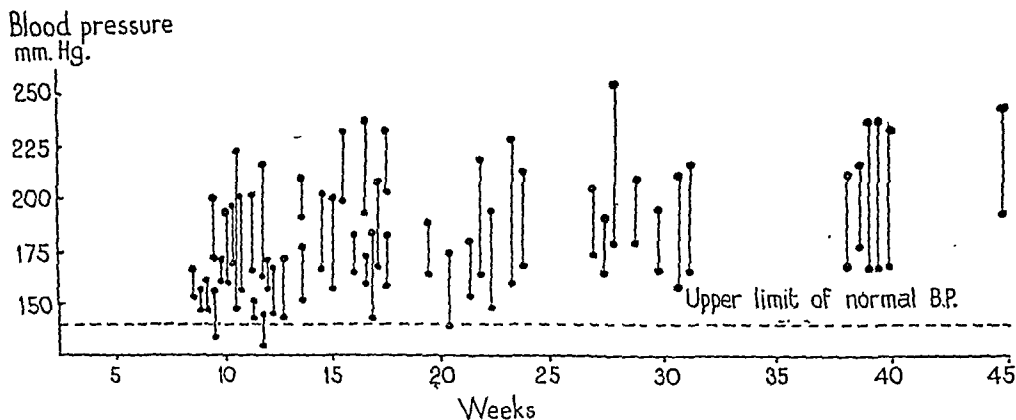


FIG. 1. Maximum (upper dot) and average (lower dot) systolic blood-pressures plotted against duration of hypertension (minimum duration, 8 weeks)

to compensatory hypertrophy of the kidney. These chronic changes bear a close resemblance to those of chronic hypertensive Bright's disease, and we have attempted to illustrate the similarity by photomicrographs of the glomeruli from the hypertensive rat on the one hand and from examples of various kinds of chronic hypertensive Bright's disease on the other (Plate 4, Figs. 3-8). As in the acute stages, the lesions are essentially focal and perivascular in distribution, occurring in wedge-shaped areas whilst the rest of the kidney tissue appears normal, but in the most severe cases the changes may be so extensive that the greater part of the kidney is involved (Plate 5, Fig. 9).

*Arteries and arterioles.* Arterial lesions in the kidney are practically confined to the interlobular arteries and glomerular arterioles. The lesions are less conspicuous than in the acute stages and are best recognized in sections stained for elastic tissue. Cellular thickening of the intima, often with irregularity and fragmentation of the elastica interna, is the characteristic lesion (Plate 5, Fig. 11), but frequently the only obvious change is an irregular, fibrous thickening of the vessel wall with distortion of the elastica interna and perivascular cuffing with small lymphocytes. It is not uncommon to find both acute and chronic lesions in the same section, that is, acute fibrinoid necrosis in some vessels and fibrous endarteritis in others. Elastic hyperplasia in the intima (elastosis) of the larger arteries, though occasionally

TABLE I

*List of Rats with Hypertension over eight weeks, Grouped in Order of  
Decreasing Severity of Lesions in the Unclamped Kidney*

Rat	Systolic blood-pressures (mm. Hg.)		Duration (weeks)
	Maximum	Average	
29 LS.	230	160	39
28 BS.	205	153	30
21 RCLDS.	235	189	46
23 RC.	190	154	29
40 BC.	225	157	22
22 RCLS.	169	159	8
28 RS.	190	145	21
29 BO.	210	160	30
29 BS.	215	162	10
40 RC.	250	174	27
43 BO.	170	156	15
45 BO.	206	165	16
47 BC.	165	144	10
24 BS.	225	162	40
30 LS.	200	155	9
36 LC.	200	164	9
37 RC.	195	168	9
45 RS.	174	149	12
30 BS.	185	160	27
37 BO.	200	164	13
42 RS.	222	145	9
48 BS.	230	197	14
57 BO.	192	160	8
39 BO.	208	166	22
26 LC.	210	171	39
30 BO.	200	168	26
39 LS.	230	160	39
40 LC.	198	171	8
48 RC.	230	200	16
52 LC.	160	150	20
47 RC.	144	127	10
21 BO.	208	187	12
22 RC.	204	174	28
35 RCLS.	180	164	15
35 BO.	186	162	18
42 LC.	198	155	13
43 BC.	155	132	8
47 RCLS.	180	155	16
48 RCLS.	170	136	19
49 BC.	235	190	15
49 BO.	215	160	20
38 LC.	205	160	39
37 LC.	182	141	15
57 RS.	170	142	11
57 BS.	150	143	10
18 RC.	158	146	8
41 RC.	170	155	10
56 LC.	155	146	8
56 BC.	165	152	8

observed in other organs (Plate 5, Fig. 12), is a rare occurrence in the kidney. In four rats, changes were found in the larger renal arteries closely resembling those seen in periarteritis nodosa; the lesions are in the subacute or chronic stage, with organized intimal thickening and periarterial fibrosis, but in two instances fibrinoid material is also seen in the vessel wall. These changes in the larger arteries are more conspicuous in the pancreas and are described in detail below. It is interesting to recall that similarly in malignant hypertension in man, fibrinoid necrosis and endarteritis are usually confined to the glomerular arterioles and interlobular arteries, but occasionally occur also in the larger arteries. In addition to the above changes, arteries of the unclamped kidney, particularly the interlobulars and the glomerular arterioles, display conspicuous and uniform muscular hypertrophy of the medial coat. No such hypertrophy was observed in the arteries of the clamped kidney (Plate 5, Figs. 13 and 14).

*The glomeruli.* The commonest glomerular lesion is a localized adhesion between the tuft and Bowman's capsule, and this is frequently accompanied by cellular proliferation in the adherent lobule of the tuft (Plate 4, Fig. 3). More extensive adhesions are common and may cause complete obliteration of the capsular space with corresponding loss of lobulation and increased cellularity of the tuft. In later stages no capillary outline can be seen, the nuclei lying in a mass of pale eosinophil, hyaline, often vacuolated material (Plate 4, Fig. 5), a condition closely resembling that described by Russell (1929) in human chronic nephritis under the term 'toxic glomerular atrophy'. In more advanced lesions glomeruli are seen in various stages of this atrophy up to complete hyalinization. In other glomeruli the capsular space is dilated, the lining epithelial cells are swollen and desquamated, and the glomerular tuft is distorted and adherent at several points (Plate 4, Fig. 7). Capsular and pericapsular fibrosis are not infrequent, but proliferation of the epithelium of Bowman's capsule is only rarely seen. In cases showing acute and chronic arterial lesions the glomeruli may also show both types of change, so that in addition to the appearances described above, acute focal necroses of the glomeruli may be present. Although glomerular lesions are most common in the wedge-shaped areas, they are also frequently found in the intervening areas of healthy-looking kidney tissue.

*The tubules.* In the wedge-shaped areas the tubules show varying degrees of dilatation, whilst other tubules are atrophied and surrounded by fibrous tissue (Plate 5, Fig. 9). In the intervening kidney tissue the tubules may appear normal or may show severe albuminous degeneration, hyaline droplet degeneration is occasionally observed, collections of neutrophil leucocytes and red blood cells may be seen in the lumen, and hyaline casts are common.

*Interstitial tissue.* Interstitial fibrosis is present in the areas of tubular atrophy and consists of proliferation of fibroblasts, deposition of collagen, and infiltration with small lymphocytes; in less severe cases slight focal lymphocytic infiltration may be the only change and this is usually concentrated round arterioles or glomeruli.

An attempt was made to discover whether the extent of the lesions in the unclamped kidney is related to the degree and duration of the hypertension. The renal damage was classified as severe, moderate, slight, or absent on the basis of a low-power survey of a whole section of the kidney. Such a method has no pretence to accuracy, but the results tallied in most cases with those obtained from counts of the glomeruli by an independent observer. Table II summarizes the relationship between the severity of the lesions in the

TABLE II

*Duration and Severity of Hypertension and Extent of Histological  
Damage in Unclamped Kidney*

Group	Lesions in unclamped kidney	Maximum blood-pressure (mm. Hg.)		Average blood-pressure (mm. Hg.)		Duration in weeks	
		Range	Over 200 in	Range	Over 160 in	Range	Over 20 weeks in
I							
18 rats	Severe	250-165	12 rats	189-145	11 rats	9-46	9 rats
II							
12 rats	Moderate	230-160	9 rats	200-145	10 rats	8-39	6 rats
III							
12 rats	Slight	235-145	5 rats	190-127	7 rats	8-39	3 rats
IV							
7 rats	Absent (or very slight)	182-150	0 rats	160-142	1 rat	15-8	0 rats

unclamped kidney and the degree and duration of hypertension. Renal damage was assessed as severe in 18 rats, moderate in 12, slight in 12, and absent in 7. The first two groups, with severe and moderate renal damage, present no appreciable difference in the degree or duration of the hypertension; the third and fourth groups, in which renal damage was slight or absent, show a definite falling off in both degree and duration of the hypertension. The fallacies involved in estimating the extent of the renal lesions and in this 'statistical' method of presenting the blood-pressure values are too great to expect any close quantitative relationship, but it is clear on the one hand that extensive lesions in the unclamped kidney are always associated with hypertension of high degree, though not necessarily long sustained; on the other hand it is equally obvious that a marked degree of hypertension may persist for many months without producing significant histological damage in the unclamped kidney. Whether this discrepancy is due to individual differences in the susceptibility of the rat to increased blood-pressure, or to different rates of development of the hypertension is not clear, but the fact emerges that neither the height nor the duration of the hypertension alone determines the production of lesions in the unclamped kidney. This failure of some rats with hypertension to develop renal changes of the type described above has a parallel in human hypertensive disease, for it is a common observation that patients with essential hypertension may

continue for many years with a very high blood-pressure and yet show no signs of developing the renal lesions which characterize malignant hypertension. Future work must decide what additional factor determines the 'malignancy' of the hypertension; our observations suggest that it may well be sudden development or sudden exacerbation of the high blood-pressure.

*Changes in the clamped kidney.* In our previous paper we stated that acute lesions such as were observed in the opposite kidney were absent from the clamped kidney, and from this fact we inferred that the lesions were hypertensive in origin. In the present series this observation was confirmed, no lesions of the type described above being found in the clamped kidney, with the exception of occasional acute glomerular necroses in the margins of areas of infarction. In the human kidney focal necroses and adhesions are similarly found in the peripheral zones of renal infarcts from non-infective emboli (Russell, 1929). There is, as we have previously stated, no relationship between the hypertension and the degree of atrophy in the clamped kidney. Thus the hypertension may be considerable and well sustained, without any microscopic abnormality being found in the clamped kidney. This is well illustrated by Plate 5, Fig. 10, a photomicrograph of the clamped kidney, for comparison with Plate 5, Fig. 9, which shows a section of the unclamped kidney of the same rat. In most cases, however, varying degrees of simple atrophy are present, this usually being moderate or severe with a corresponding contraction of the kidney. The atrophy is diffuse and involves the tubules more than the glomeruli; in extreme instances tubules can no longer be recognized, whilst the glomeruli are small and closely packed, but otherwise normal in appearance. This picture is in striking contrast to the focal fibrosis, with grossly abnormal arteries and glomeruli, observed in the unclamped kidney. In many cases the clamped kidney has undergone partial infarction which may perhaps be due to operative manipulation. It is of no significance in relation to the hypertension, except that when complete infarction is produced no hypertension follows. It is worthy of note that in rats with considerable and persistent hypertension the clamped kidney is occasionally found to be grossly contracted, infarcted, and calcified, whilst histologically the damage is so severe as to make it unlikely that any excretory function remains.

*Lesions in other organs.* In our previous paper we described acute vascular lesions in the pancreas, mesentery, intestines, stomach, suprarenals, liver, and testis. Acute focal necrosis of the parenchyma was also described in the heart, pancreas, and liver. These parenchymal lesions, examples of which are illustrated in Plate 6, Figs. 15 to 17, are occasionally seen in malignant hypertension in man. In the present series of rats with long-standing hypertension chronic arterial lesions were likewise found in these organs, and resemble those present in the kidney. In the heart fibrous scarring of the myocardium is almost invariably present and predominantly affects the right ventricle; the fibrosis is usually perivascular and when superficial is seen by

the naked eye as pearly white, leaf-shaped scars under the pericardium. Vascular changes are confined to the small arteries and closely resemble those in the kidney, except that intimal fibrosis is more common; acute fibrinoid lesions are sometimes superadded, and are associated with perivascular necrosis of the myocardium. Cardiac hypertrophy is well marked in rats with long-standing hypertension, and particularly affects the left ventricle. The pancreas shows most striking lesions in the branches of the mesenteric artery which pass through and supply it, and the changes were observed in 32 out of 47 rats examined. Relatively large arteries are often involved and lesions are seen in acute, subacute, and chronic stages, closely resembling those of periarteritis nodosa. To the naked eye the lesions in the acute stage present a remarkable picture with nodular bluish swellings of the branches of the mesenteric arteries (Plate 6, Fig. 18). The earliest change is the appearance of a homogeneous fibrinoid or hyaline substance between the elastica interna and the endothelium (Plate 6, Fig. 19). Although the fibrinoid substance may be confined to the intima, it is occasionally seen in both intima and media, or in the media alone. Erythrocytes and neutrophil leucocytes may be present in the wall, whilst the adventitia becomes infiltrated with acute inflammatory cells, neutrophil leucocytes, eosinophil leucocytes, plasma cells, and large mononuclear cells. The elastica interna is greatly stretched and frequently ruptured, whilst occasionally only fragments of elastic tissue may be visible. Nodes of periarteritis are often seen at intervals along an artery where small branches are given off (Plate 6, Fig. 20) and true aneurysm formation is common. In the subacute stage the intima becomes cellular, cells appearing first in the inner zone near the endothelium; small lymphocytes and fibroblasts appear in the adventitia, and a broad zone of perivascular granulation tissue develops. In the later stages fibrosis develops and commonly produces an eccentric thickening of the intima, in which proliferation of elastic tissue may be present (Plate 5, Fig. 12); all layers of the vessel wall are involved and dense collagen fibres are deposited in the perivascular tissue. Acute fibrinoid lesions and chronic organized changes are frequently present in the same section, and occasionally in the same vessel, suggesting a fresh lesion in an artery previously damaged. We have observed acute lesions in rats killed four days after clipping one renal artery, but on the other hand such lesions have been found in animals which have had persistent hypertension for nine months, indicating that recurrent attacks of acute vascular damage occur apparently throughout the course of the disease.

These lesions of the mesenteric arteries have led to fatal complications in several instances; on post-mortem examination intestinal obstruction was found in four rats, perforation of the gut and peritonitis in one, haemorrhage into the gut in two, and retroperitoneal haemorrhage in another. In several rats distension of the abdomen by enlarged mesenteric vessels was so gross as to simulate pregnancy. If the clamped kidney is removed and the blood-pressure returns to normal the rat appears to have extraordinary powers of

recovery from this severe arteritis of the mesenteric vessels; for example, in one rat (no. 49 BO) laparotomy revealed extensive periarteritis nodosa similar to that shown in Plate 6, Fig. 18, but at autopsy 12 weeks later there was no sign of any macroscopic lesion in the pancreas, although microscopically fibrosis of all coats of the arteries with marked periarterial fibrosis was present. The spontaneous occurrence of arterial lesions closely resembling those described above has been observed (Wilens and Sproul, 1938) in rats over the age of 500 days. The animals used in our investigation were well below this age and we have observed no such lesions in control animals which failed to develop hypertension.

Apart from these findings in the heart and pancreas, arterial changes in other organs are as a rule inconspicuous unless acute lesions have been super-added; cellular intimal thickening was occasionally seen in small arteries in the intestine, stomach, and liver. Finally in several rats which died, usually after a sudden rise in blood-pressure, post-mortem examination revealed gross anasarca, including oedema of the lungs, pancreas, and mesentery, large pleural effusions and ascites, and these changes were associated with central congestion of the liver. Such indications of terminal myocardial failure are interesting in view of the frequency and severity of the lesions in the myocardium.

Our main conclusions from the above observations are (1) in rats with sustained hypertension produced by constricting one renal artery, chronic lesions of considerable severity may be present in the opposite kidney, and the extent of these is related to the severity of the hypertension, (2) from the predominant involvement of the arteries and glomeruli, and the reduction in number of the latter, this damage, if sufficiently severe, might be expected to cause a diminution in blood-flow through the kidney, and (3) these chronic lesions closely resemble those found in various types of chronic hypertensive Bright's disease. This resemblance is most exact to malignant hypertension, particularly to those cases which have run the full course to malignant nephrosclerosis. The detailed description of changes in the arteries, glomeruli, tubules, and interstitial tissue of the rat's kidney can be applied almost verbatim to the kidney of malignant hypertension. In other types of chronic hypertensive Bright's disease, for example in chronic nephritis, we cannot expect to see hypertensive lesions alone, since these would be superimposed on changes due to the original kidney disease. It may, therefore, be difficult to recognize how much damage is due to the hypertension. This question will be dealt with more fully below, but it is obvious from the above description that certain chronic changes in the glomeruli, tubules, and interstitial tissue which occur in chronic nephritis in man are also found in the unclamped kidney of the hypertensive rat.

#### *Effect of Excision of the Clamped Kidney on the Hypertension*

Several observers have shown that in dogs (Goldblatt, 1937; Blalock and Levy, 1937; Dicker, 1937) where hypertension has been produced by con-



stricting both renal arteries or by constricting one renal artery and removing the opposite kidney, release of the clamps or excision of the single clamped kidney has resulted in rapid disappearance of the hypertension. Our experiments differ from the above in that they afford an opportunity of studying the effect of a long-sustained hypertension on an unclamped kidney. We have described above the structural changes found in the unclamped kidney. If these changes are themselves capable of giving rise to hypertension we might expect that in some animals the blood-pressure would remain elevated after removal of the clamped kidney. To decide this question we have excised the clamped kidney in 27 rats with varying degrees of hypertension and at different intervals after constriction of the renal artery. In some instances the kidney has been excised after laparotomy had revealed a granularity of the unclamped kidney which we have found to indicate fairly extensive chronic lesions of the type described above; in others the clamped kidney was removed as a therapeutic measure during a 'vascular crisis'. Finally, the clamped kidney was excised in six control rats which had failed to develop hypertension after constricting one renal artery.

Results are given in Table III and characteristic blood-pressure curves before and after excision of the clamped kidney are shown in Fig. 2 (*a*, *b*, and *c*). It is apparent that hypertension persists after removal of the clamped kidney in many instances. Such 'residual' hypertension has usually been maintained until the rat has been killed for histological examination, the longest period in the series being 21 weeks. Hypertension probably persists after nephrectomy more often than appears from the table for a number of reasons. Firstly, the method of tabulation is unsatisfactory since, as previously explained, average and maximum blood-pressure values may give an inaccurate impression of the blood-pressure level throughout the experiment. Secondly, the condition of the rat may be poor after removal of the clamped kidney owing to the persistent effects of a vascular crisis, to post-operative infection, or to uraemia, and any of these conditions may depress the blood-pressure. Thus, in 16 animals the clamped kidney was removed during a vascular crisis, and five of these animals failed to improve and died without exhibiting any hypertension; in three rats with prolonged depression of the blood-pressure after nephrectomy, chronic pneumonia was found *post mortem*; uraemia developed in two instances, the blood-urea rising to 90 and 140 mg. per 100 c.c. Thirdly, in an ill rat the ether anaesthesia may depress the blood-pressure during the determination, so that deceptively low readings may have been obtained in any of the three conditions just described.

In view of these factors it is obvious that any conclusions must be based on a detailed study of the course of the experiment in each rat individually. For purposes of description we have divided the results into five groups (Table III):

*Group 1.* Contains four rats with a high level of blood-pressure, lasting from 6 to 41 weeks before nephrectomy, in which the general level of

TABLE III. *Effect on Hypertension of Removal of Clamped Kidney*

Rat	Blood-pressure (mm. mercury)				Renal lesions		
	Before excision		After excision		Unclamped kidney		Clamped kidney
	Maximum	Average	Duration in weeks	Maximum	Average	Duration in weeks	Low-power estimate of renal damage
							Abnormal glomeruli in a complete sagittal section
							Number
							%
							Degree of atrophy
21 ROLDS.	235	188	41	215	191	6	5
45 LS.	240	193	12	280	183	21	44
46 RC.	182	144	8	170	144	10	68
40 BC.	180	180	6	172	156	14†	96
20 LS.	230	167	32	160	111	7†	40
24 BS.	225	170	30	155	141	10	32
26 LC.	210	170	28	156	130	11	22
40 RC.	250	183	20	185	142	7†	21
42 RS.	225	164	5	150	137	15	13
39 LS.	225	210	9	170	120	12	13
49 BO.	215	178	10	148	130	12	13
52 LC.	148	142	3*	160	144	18	15
47 BC.	165	144	10	148	130	10†	16
45 BS.	158	119	3*	145	123	12	15
42 BC.	172	146	5	144	111	4*	18
44 LC.	128	122	4	148	125	12	13
38 LC.	205	160	15	146	124	12	9
48 RCLS.	170	132	10	148	138	9	3
41 RCLS.	205	170	7	122	111	4*	13
39 BO.	208	166	22	125	102	5	8
40 LC.	198	153	7	135	114	12*	8
41 BC.	172	120	5	135	109	9*	11
43 RC.	170	162	6	120	94	9*	22
43 BO.	170	157	17	130	122	6	3
42 LS.	150	114	6	134	106	12*	5
44 RC.	148	125	5	90	86	3†	
44 BO.	145	130	4	136	117	7	
49 RC.	120	86	5	115	90	8	
49 LC.	138	122	10	135	124	9	
46 RS.	110	98	5	138	102	8	
56 RC.	138	121	9	130	122	5	
58 LCRS.	120	105	5	125	121	6	
44 RS.	126	109	4	120	102	6	

\* Severe constitutional disturbance (vascular crisis) with prolonged depression of blood-pressure, before or after nephrectomy.  
† Postoperative pneumonia.

hypertension was maintained after nephrectomy. In one rat the hypertension persisted for 21 weeks after nephrectomy with an average blood-pressure of 183 mm., until the animal had unfortunately to be destroyed at the outbreak of war.

*Group 2.* Contains five rats with high initial blood-pressures continuing

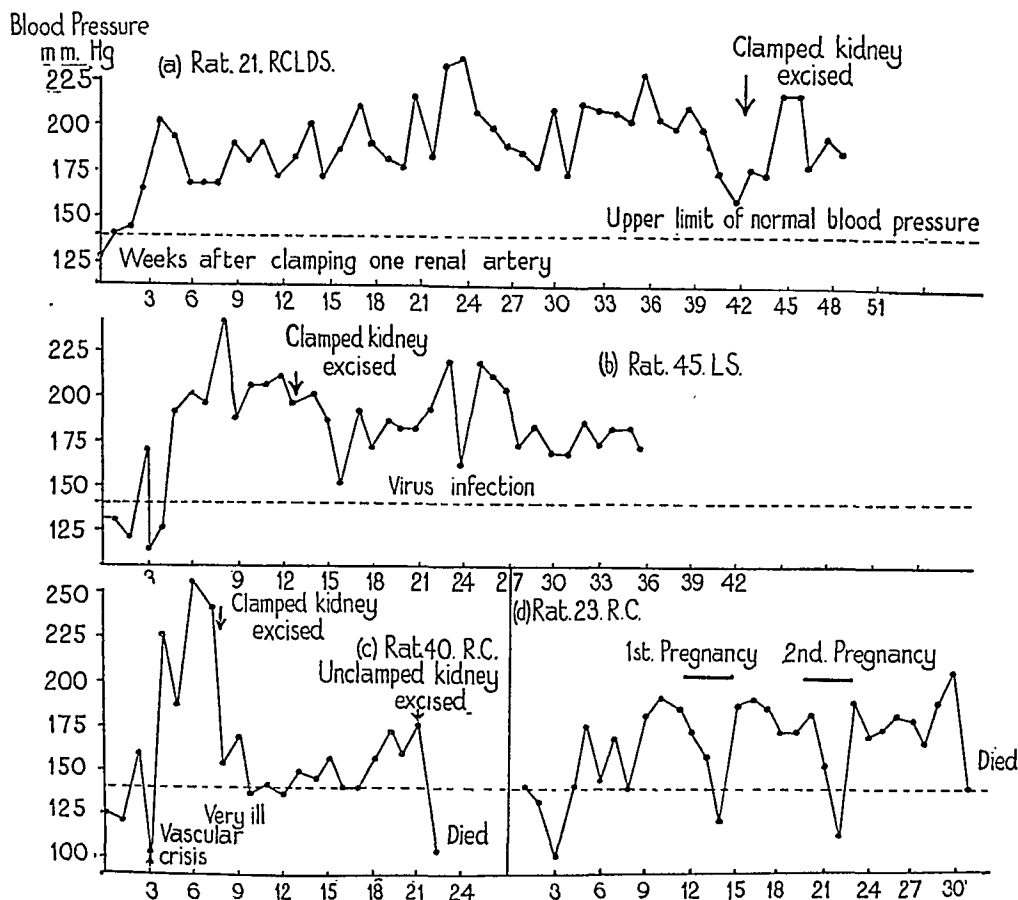


FIG. 2

for periods up to 30 weeks; in this group the hypertension persisted after nephrectomy, but at a lower level.

*Group 3.* Consists of nine rats with considerable to moderate hypertension before the operation and intermittent hypertension (two or more readings over 140 mm.) after it. In one animal with two postoperative readings over 140 mm. the maximum recorded blood-pressure during the four weeks before operation was 128 mm. As there were definite lesions in the unclamped kidney, we can explain this exceptional finding only by the assumption that a short period of hypertension occurred between our weekly determinations of blood-pressure before nephrectomy.

*Group 4.* Contains nine animals with hypertension of varying degree before nephrectomy and no readings above 140 mm. after the operation.

At first sight it is surprising that with hypertension of such a height and duration as displayed by many members of this group, there should be no residual hypertension after removal of the clamped kidney. We have previously noted, however, that a marked hypertension may persist for months without producing significant damage in the unclamped kidney, so that the above result is to be expected if persistence of hypertension after removal of the clamped kidney is determined by the presence of damage in the unclamped kidney.

*Group 5.* Contains six control animals in which the blood-pressure before and after nephrectomy was below 140 mm.

It will be seen that the series presents a gradation of response rather than a clear separation into groups. The main fact so far established is that after removal of the clamped kidney the hypertension may persist at a high level for many months.

#### *Relation of the Residual Hypertension to the Lesions in the Unclamped Kidney*

We have in the present experiments no direct proof that the hypertension which persists after removal of the clamped kidney is maintained by the unclamped kidney. Subsequent removal of the remaining kidney in one rat has resulted in a prompt fall in blood-pressure from 172 mm. to the normal level (Text-Fig. 2, *c*). Any conclusions drawn from blood-pressure changes after removal of both kidneys must, however, be open to question, and we have not pursued this line of inquiry. Again, we have no method of determining the extent to which the vascular lesions in the unclamped kidney reduce the blood flow through it. We can, therefore, produce only indirect evidence that the residual hypertension is due to ischaemic changes in the unclamped kidney. With this object we have correlated in the five groups described above the degree of residual hypertension with the amount of renal damage, which we estimated by low-power examination, supplemented by counting the abnormal glomeruli in a complete sagittal section of the kidney. The total number of ischaemic nephrons in the kidney might be expected to correspond fairly closely with the number of abnormal glomeruli. Whether hypertension due to renal ischaemia depends on the absolute number or the percentage of ischaemic nephrons is uncertain; we have therefore given both the number and percentage of abnormal glomeruli in a complete sagittal section (Table III, columns 9 and 10).

It will be seen that there is a fairly constant relationship between the amount of residual hypertension and the severity of the lesions in the unclamped kidney. If we compare the hypertension with the number of abnormal glomeruli the correspondence is even closer. There are some discrepancies but, excluding those rats in which there was a postoperative depression of the blood-pressure due to the extraneous factors mentioned above, only very occasional cases show a poor correlation. Such a result is the more remarkable when we consider the fallacies involved in deducing functional changes from histological lesions; moreover there is a risk of

considerable error in taking a single sagittal section as representative of the whole kidney.

We may summarize these observations as follows :

1. Gross residual hypertension was always associated with extensive lesions in the unclamped kidney.
2. Moderate residual hypertension, in the absence of complications, occurred in animals with a moderate degree of renal damage.
3. In no case with extensive lesions (over 25 per cent. of abnormal glomeruli) did the blood-pressure return to normal after nephrectomy.
4. With lesser degrees of renal damage residual hypertension was intermittent or absent, but in the majority of cases showing no hypertension after nephrectomy there was some complicating factor leading to prolonged depression of the blood-pressure.
5. In the control group, in which no hypertension had resulted from clamping one renal artery and in which lesions were absent in the unclamped kidney, the blood-pressure was invariably normal after removal of the clamped kidney.

These facts strongly suggest that the residual hypertension is caused by the damage in the unclamped kidney. Absolute proof is at present unfortunately lacking, for it is conceivable, though unlikely, that arterial lesions in other organs may cause sufficient increase in peripheral resistance to account for the persistent hypertension. We have found no correlation between the extent of arterial lesions in other organs and the residual hypertension; in rat 52 LC. for instance, which had residual hypertension up to 160 mm. for 18 weeks after removal of the clamped kidney, the only lesions observed in organs other than the kidney were slight scars in the myocardium, whilst the arteries of the pancreas and mesentery, which are the sites of election for vascular changes, showed no abnormality.

#### *Can the Residual Hypertension Produce Further Renal Damage?*

We hoped to obtain an answer to this question from the histological changes in the unclamped kidney of rats with prolonged residual hypertension after removal of the clamped kidney. If recent lesions, in particular acute fibrinoid necroses of the renal arterioles and glomeruli, are discovered many months after removal of the clamped kidney it may be presumed that these were produced by the residual hypertension. Although we have no method of discovering how long acute fibrinoid lesions may persist, we observed in our earlier experiments the development of organized lesions within three weeks of the onset of the hypertension. Furthermore, if the residual hypertension can produce progressive renal damage we might expect to see the vicious circle run to its inevitable termination with the eventual development of renal failure. The investigation of this question was abruptly terminated by the outbreak of war when our experimental animals unfortunately had to be destroyed. From the material available we have several examples of acute fibrinoid necrosis of arteries and arterioles in the kidney or in other organs

of rats which died from two to four months after removing the clamped kidney.

*Rat 29 LS.* Hypertension up to 230 mm. for 32 weeks after clamping right renal artery; removal of right kidney followed by residual hypertension up to 160 mm. for 7 weeks, rat then killed; kidney shows gross damage with chronic lesions in arteries and glomeruli as described above, and in addition fibrinoid material in the walls of many arteries and arterioles and acute necrotic changes in many glomeruli.

*Rat 24 BS.* Hypertension up to 225 mm. for 30 weeks; removal of clamped kidney followed by residual hypertension up to 155 mm. for 10 weeks; acute fibrinoid necrosis of arterioles in testis.

*Rat 42 RS.* Hypertension up to 225 mm. for 5 weeks; removal of clamped kidney followed by residual hypertension up to 150 mm. for 15 weeks; acute arterial lesions in myocardium.

#### *Additional Experiments*

*Effect of removal of the clip from the renal artery followed by excision of the unclamped kidney.* In four rats in which sustained hypertension at a high level followed compression of one renal artery, the clip on the artery was subsequently removed. The results are shown in Table IV. In all four rats

TABLE IV

*Effect of Removal of Clip on Course of Hypertension and Histological Changes in Kidneys*

Rat	Before removal of clip		After removal of clip		'Hypertensive' Renal Lesions	
	Duration of hyper- tension in weeks	Maximum blood- pressure (mm. Hg.)	Duration of hyper- tension in weeks	Maximum blood- pressure (mm. Hg.)	Unclamped kidney	Clamped kidney
47 RCLS.	14	180	5*	170	++	+
48 RC.	14	230	7*	170	+++	+
48 BS.	14	225	7	144	slight	few arterioles
49 BC.	14	235	6	148	slight	focal cortical necrosis

\* Unclamped kidney removed after 3 weeks.

the blood-pressure fell after removal of the clip, but some residual hypertension remained. This was most marked in the first two, rats 47 RCLS. and 48 RC., where readings up to 170 mm. were observed. In an attempt to show that this residual hypertension was due to lesions in the unclamped kidney the latter was excised, in both cases three weeks after removal of the clip. In rat 47 RCLS. the blood-pressure was still 170 mm. one week later, then fell to 125 mm.; in rat 48 RC. the four weekly readings after excision of the unclamped kidney were 140 mm., 145 mm., 150 mm., and 145 mm., that is, persistent hypertension, but at a lower level. The most probable explanation of these results is that the 'ischaemia' of the clamped kidney was not entirely relieved by removal of the clip, for in both rats typical wedges of ischaemic atrophy were found in the clamped kidney; furthermore, it is possible that

some constriction of the renal artery might still be present owing to the periarterial fibrosis always found in the region of the clip.

An interesting histological finding in these rats was the presence in the clamped kidney of occasional lesions in the arterioles and glomeruli such as we had previously encountered only in the unclamped kidney. These we attribute to the effect of the residual hypertension on the clamped kidney after removal of the clip, and their occurrence confirms our previous assumption that the clamped kidney is normally protected from the effects of the hypertension by the clip on the renal artery, and is further evidence that the lesions are hypertensive in origin. Arterial and glomerular lesions were present also in the unclamped kidneys of these four rats, and in all cases were much more extensive than in the corresponding clamped kidneys. In one rat (49 BC.) the previously clamped kidney showed areas of acute cortical necrosis, a lesion we have occasionally observed in unclamped kidneys.

*Removal of the opposite kidney after clamping one renal artery.* In 15 rats a clip was placed on one renal artery and at a later date the opposite kidney was excised, after inspection of the clamped kidney had shown the absence of any gross macroscopic damage. Eleven of these rats had had hypertension for periods of five to 16 weeks before nephrectomy, whilst the remaining four had been followed from four to 14 weeks and showed no hypertension. The results of this experiment were as follows. In the four rats with no previous hypertension the blood-pressure remained normal after excision of the unclamped kidney. One of the eleven rats with previous hypertension developed postoperative pneumonia and died one week later with a normal blood-pressure; in the remaining 10 the blood-pressure rose to a higher level after removal of the unclamped kidney. Six of these 10 died after intervals varying from six to 13 weeks, uraemia being obviously the cause of death in four and probably in a fifth; the sixth developed a virus infection which proved fatal; the remaining four were killed at varying intervals. Histological examination of the kidneys revealed changes of considerable interest. The unclamped (excised) kidneys displayed lesions such as we have previously described, varying in extent according to the degree of hypertension. The clamped kidneys appeared normal in size or slightly atrophied; in five rats there were no histological changes apart from slight cortical atrophy such as is regularly seen in the clamped kidney; the other five had lesions in the clamped kidney of the same kind as we have previously described in the unclamped kidney. These consisted for the most part of organized glomerular adhesions and necroses, occasionally foci of tubular dilatation were present, and in one rat there was gross tubular dilatation with the typical picture of chronic interstitial nephritis. Our interpretation of these findings is that in these animals excision of the unclamped kidney produced a sudden rise in intravascular pressure in the clamped kidney. This might occur either owing to the increased blood-pressure, which frequently followed immediately upon excision of the unclamped kidney, partly overcoming the resistance of the clip, or, assuming

the constriction of the renal artery to be such that little or no increase in blood-flow could take place, a sudden addition to the load on the clamped kidney could be dealt with only by raising the filtration pressure in the glomeruli; this in turn could be brought about only by constriction of the efferent arterioles with a resultant increase in intra-arterial pressure distal to the clamp. The first possibility is supported by the following facts. When the blood-pressure was normal after removal of the unclamped kidney (whether hypertension was previously present or not) lesions of this type were absent from the clamped kidney; again there was a distinct proportionality between the hypertension before nephrectomy and the lesions in the unclamped kidney on the one hand, and between the hypertension after nephrectomy and the lesions in the clamped kidney on the other. For example, rat 43 BC. had slight hypertension before operation (maximum 155 mm.) and the unclamped kidney showed only occasional glomerular lesions; immediately after nephrectomy the blood-pressure rose to 185 mm. and the clamped kidney showed the gross lesions to which we have already referred. If these latter changes had been produced before removal of the unclamped kidney we should expect similar changes to be present in that kidney instead of the inconspicuous lesions which were actually found.

It might be suggested that renal insufficiency played some part in the production of these lesions in the clamped kidney, but our observations do not support this view. In one rat which died in uraemia the clamped kidney showed no lesions, whilst in two rats with fairly numerous lesions there was no evidence of uraemia, and the animals remained apparently healthy until they were killed for histological study. The finding in these experiments of lesions in the clamped kidney similar to those previously seen only in the unclamped kidney came to us as a surprise and is difficult to explain satisfactorily. We feel, nevertheless, that in view of the peculiar nature of the experiment this finding is not inconsistent with our interpretation of the hypertensive origin of the lesions. The significant fact is that such lesions were never found in the clamped kidney so long as the opposite kidney was left intact.

*Effect of pregnancy on experimental hypertension in the rat.* During the course of our experiments five rats in which hypertension had been produced by clamping one renal artery became pregnant, and in all cases a marked fall in blood-pressure occurred. Although occasional low readings of the blood-pressure may be encountered in any rat, due to causes previously mentioned, the regularity of this phenomenon led us to the conclusion that the fall in blood-pressure was due to the pregnancy. This seemed probable also because the blood-pressure curve often showed a progressive fall and subsequent return to its high level extending over two to three weeks. Furthermore in one rat, the first pregnancy was followed after an interval of six weeks by a second pregnancy, and the blood-pressure underwent an almost identical fall (Fig. 2, d). In all five rats the blood-pressure fell below the normal level, the maximum fall being from 180 mm. to 80 mm.



The animals showed no untoward symptoms and after labour the pressure rose again to its previous level. The fall in pressure usually occurred during the latter half of gestation and its constancy led us to diagnose pregnancy in the later cases before any other signs had been noted. We have been unable to pursue this investigation further and can offer no explanation of the fall in blood-pressure. From our observations in these rats it is difficult to say what was the effect, if any, of pregnancy on the subsequent course of the hypertension. Three of the rats subsequently died following a peak of hypertension higher than any observed before pregnancy, but the intervals between pregnancy and death were 15, 12, and 9 weeks, during which time the animals appeared to be in perfect condition. Again, although acute arterial lesions were found *post mortem* in the unclamped kidney and in other organs, these were neither more severe nor more extensive than in the series as a whole.

### *Conclusions*

In the introduction we outlined the conception of a vicious circle arising from the effect of hypertension on the kidney. This conception was based on two observations, firstly the fact, demonstrated by Goldblatt, Lynch, Hanzal, and Summerville (1934), that reduction of blood-flow through the kidney in animals gives rise to hypertension, and secondly, our discovery that such hypertension, produced by partial constriction of one renal artery in the rat, gives rise to severe acute vascular lesions in the opposite kidney. We enumerated the steps by which the existence of this vicious circle might be established and our observations may be summarized as follows:

1. It has been shown that when long-sustained hypertension is produced in the rat by partial occlusion of one renal artery, chronic lesions are found in the opposite kidney. These lesions are not present in the clamped kidney, from which we infer, as in the case of the acute lesions previously described, that they are the result of the hypertension, the absence of the lesions from the clamped kidney being attributable to the 'protective' action of the clamp on the renal artery. Levy, Light, and Blalock (1938) have shown that in dogs with experimental hypertension produced by clamping the renal arteries there is a decrease in arterial pressure distal to the clamp. Although we have no direct proof in our animals that there is no hypertension distal to the clip, we have produced indirect evidence in that the arteries of the unclamped kidney show great medial hypertrophy, whilst those of the clamped kidney show none, and when the clip is removed and hypertension persists, lesions are found in the previously clamped kidney.

2. The character and extent of these lesions are such as might be expected to reduce the blood-flow through the affected parts of the kidney. In the acute stages the lesions are predominantly vascular, involving arteries, arterioles, and glomeruli with associated damage to the renal parenchyma. The chronic changes are such as we should expect from organization of these acute lesions, and the fact that this process is seen in different stages in the same section suggests its progressive nature. The extent of the renal damage

varies in different animals and, although essentially focal in distribution, it may be so extensive as to involve the greater part of the kidney tissue.

3. We have emphasized and illustrated the histological identity of the lesions with those of chronic hypertensive Bright's disease. In our previous paper we demonstrated the striking resemblance of the acute lesions with those encountered in malignant hypertension. The chronic lesions here described are perhaps less striking, but their close resemblance to those found in chronic hypertensive Bright's disease throws on the origin of the histological changes in the kidney in this group of disorders a new light which has far-reaching clinical implications. In cases of essential hypertension Russell (1929) described 'ischaemic changes' and McGregor (1930) described 'hypertensive' changes in the glomeruli. Both writers drew a clear distinction between these lesions and changes of an 'inflammatory' type. The lesions we are discussing in hypertensive Bright's disease are in effect those of the 'inflammatory' type and should not be confused with the 'ischaemic' or 'hypertensive' glomeruli of the above authors.

4. It has been shown that hypertension may persist after removal of the clamped kidney and that such persistent hypertension usually lasts until the animal is killed, the longest period in the series being 21 weeks. This experiment was performed on 27 rats with hypertension and on six rats without hypertension as controls. In 18 of the 27 rats there was some degree of residual hypertension, this being severe in four, considerable in five, and slight in nine, despite the fact that certain complications militated against a positive result.

5. Evidence is produced correlating the hypertension which persists after removing the clamped kidney with the extent of the lesions in the unclamped kidney as estimated by low-power examination of the sections and by counting the number of damaged glomeruli. This analysis shows that the blood-pressure invariably returns to normal after excision of the clamped kidney in those rats which *post mortem* show no significant damage of the unclamped kidney, and that residual hypertension is invariably associated with structural disorganization of the unclamped kidney, the greater degrees of residual hypertension corresponding with the more extensive renal damage. This correlation strongly suggests that the residual hypertension is caused by the damage in the unclamped kidney.

6. Finally, evidence has been obtained which indicates that the residual hypertension may itself give rise to lesions in the remaining kidney. The most convincing proof of this last event in the vicious circle would be the ultimate development of renal failure due to progressive destruction of the unclamped kidney. Our investigations were abruptly terminated before we had obtained a complete answer to this question, but we have found acute arterial lesions in both the unclamped kidney and in other organs some months after the clamped kidney was removed. Since we have observed organized arterial lesions in the kidneys after three weeks of hypertension, it appears unlikely that the acute fibrinoid changes would persist for months.

We regard this as presumptive evidence that the residual hypertension can itself produce lesions in the remaining kidney.

From this chain of evidence we conclude that, in the rat, experimental hypertension produces progressive vascular lesions in the kidney, that these lesions may themselves give rise to hypertension, and that a vicious circle is thereby established which leads to sustained elevation of the blood-pressure and progressive renal destruction.

### *Clinical Implications*

*Malignant hypertension.* In our previous paper we discussed at length the controversial question of malignant hypertension. The conception of a form of essential hypertension which runs a malignant course to end in renal failure is foreign to Allbutt's clinical teaching. It has found scant recognition in the English literature, where the condition still masquerades unhappily as 'chronic interstitial nephritis'. Nevertheless, as we pointed out in the introduction, clinical and pathological evidence has lately appeared which supports this conception. The histological identity of the acute renal lesions occurring in the hypertensive rat with those which are invariably found in the kidney in malignant hypertension provides experimental evidence in support of the view that in this disease the renal lesions are secondary to the hypertension. Our present experiments have shown that after prolonged hypertension the renal damage may be so extensive as to reproduce faithfully in the rat this type of chronic interstitial nephritis. In both the human being and the rat the renal damage is predominantly focal and the production of alternating areas of tubular dilatation and atrophy is characteristic. This focal distribution is, then, an indication of the vascular origin of the renal changes.

If the acute changes in the kidney of the hypertensive rat have thrown light on the origin of the renal damage in malignant hypertension, the conception of the vicious circle is no less essential to our proper understanding of the course of that disease. It explains why the development of renal failure is more rapid in this condition than in any others, for it is not uncommon to see the renal function tests fall from normal values to those of gross insufficiency within a few weeks. It explains why, in spite of the rapid downhill course, the blood-pressure is well sustained and may even show a terminal rise. It explains the not infrequent remissions followed by relapses in which hypertensive symptoms are aggravated and renal efficiency deteriorates. These relapses are comparable with the grave constitutional disturbances which we observed in the rat and are similarly associated with wasting, convulsions, and coma. Finally, it explains the histological finding of severe acute fibrinoid necroses of arterioles and glomeruli superimposed on more chronic organized lesions.

It must be emphasized that our observations give no clue to the primary cause of the hypertension in this disease, nor do they explain why the majority of cases of essential hypertension run a benign course without

leading to renal failure. We can say, however, that when the disease enters its rapidly progressive course it is highly probable that the essential hypertension is reinforced by a renal hypertension derived from the vascular damage in the kidneys. The question arises whether up to this point the condition differs in any way from benign hypertension, that is, is malignant hypertension 'malignant' from the start. We can make only the following observations on this point:

1. As a rule malignant hypertension occurs in younger subjects than benign hypertension, and there is usually no reason to suspect a previous longstanding elevation of the blood-pressure. It appears that malignant hypertension arises *de novo* in these cases.

2. Occasionally malignant hypertension occurs in patients who have been under observation for many years as cases of benign hypertension. Here the condition appears to be a 'malignant termination' of a pre-existing hypertensive state such as we have already described in other forms of Bright's disease.

There is a second and more fundamental question, namely, is malignant hypertension renal in origin or is it primarily due to some extrarenal factor? There is both clinical and pathological evidence on this point. We have in several instances made the diagnosis of malignant hypertension (on the finding of papilloedema) before the occurrence of albuminuria or renal impairment, and at autopsy in such cases the kidneys may show no histological lesions apart from very occasional acute fibrinoid necrosis of the arterioles (Ellis, 1938). We conclude therefore that malignant hypertension exists as such before the renal changes develop, and that the malignant character of the disease is primarily determined by an extrarenal factor. The nature of this factor is unknown, but some indication may be derived from the observation that malignant hypertension occurs in pituitary basophilism (MacMahon, Close, and Hass, 1934), and that in this disease the rapid development of the malignant type of hypertension has been observed (Ellis, 1938). Certain authors imply or maintain on the other hand that the work on experimental hypertension in animals provides evidence for the renal origin of essential hypertension. Goldblatt (1938) reported that when hypertension is produced in dogs by constriction of both renal arteries sufficiently severe to produce uraemia, acute necrotic arterial lesions are found in various organs, but are not present in the kidneys. He described this as the experimental production of the malignant phase of essential hypertension and concluded that the acute arterial lesions are due to the combined effects of hypertension and uraemia. Such an interpretation assumes firstly that acute necroses of the renal arterioles in malignant hypertension are preceded by uraemia. The available clinical and experimental evidence supports the opposite view, namely that renal destruction and uraemia are caused by the acute arterial lesions. As we have already pointed out, studies on early cases of the disease have shown that arteriolar necroses appear in the kidney before the development of renal failure,

a conclusion which is supported by our observations that in the rat uraemia plays no part in the production of these lesions. Secondly, Goldblatt's view assumes that malignant hypertension is caused by a primary obstruction to the renal blood-flow analogous to the clamps on the renal arteries of the dog. There is, however, no evidence of such primary renal obstruction in malignant hypertension. Moritz and Oldt (1937) state that sclerosis of the arterioles or arteries is present in the kidneys in all cases of essential hypertension and maintain that this, by producing ischaemia, causes the hypertension. Such evidence cannot be regarded as satisfactory for it can equally well be claimed that the chronic arterial and arteriolar degeneration is the result of the hypertension (Turnbull, 1915).

In conclusion, it is probable that malignant hypertension has in its later stages a renal component, as we have attempted to show. It is possible that benign hypertension involves a renal mechanism, i.e. that a primary extrarenal factor may cause functional renal ischaemia with resulting elevation in blood-pressure, although there is so far no evidence for this; but the contention that 'essential' hypertension is due to primary disease of the kidney not only lacks experimental support, but runs contrary to our knowledge of the clinical and pathological features of the disease.

*Other forms of chronic hypertensive Bright's disease:* (a) *The malignant termination.* The demonstration that hypertension can produce necrosis of the renal arterioles and glomeruli provides the explanation of another difficulty, namely the occurrence of these acute renal lesions in other types of chronic Bright's disease with hypertension, particularly in undoubted cases of subacute and chronic nephritis. This occurrence was most confusing and contributed more than anything else to the lack of recognition of malignant hypertension by the pathologist, since it made the differential diagnosis of this condition in its later stages difficult or impossible. When it is realized that these acute vascular lesions are specific not for any one disease, but only for the hypertension which may be common to all, this objection does not arise. Thus the vicious circle may be set in motion, not only by essential hypertension, but by hypertension resulting from primary disease of the kidney. The result in both cases is a sudden deterioration in the condition of the patient with rapid development of renal failure. This malignant termination is characterized clinically by excessively high blood-pressure, hypertensive cerebral attacks (headache, convulsions, and blindness), and hypertensive retinitis with papilloedema and retinal exudates, and histologically by the occurrence of acute fibrinoid necroses of the renal arterioles and glomeruli and of similar vascular lesion in other organs.

The malignant termination can so dominate both the clinical and histological pictures that it may be impossible in the later stages to recognize the original nature of the disease. We have observed this terminal picture in cases of chronic nephritis, chronic pyelonephritis, hypertensive renal disease following toxæmia of pregnancy, and the chronic hypertension of leadworkers.

The term 'malignant hypertension' is now generally applied to the malignant form of essential hypertension and serves to bring it into contrast with the more common benign form (Allbutt's 'hyperpiesia'). We suggest that this usage be retained, rather than that the meaning be altered to embrace other types of Bright's disease with a malignant termination, for in these conditions the termination is no more than an incident in the disease process, and to call this 'malignant hypertension' only confuses the issue which we are attempting to simplify.

(b) *The progressive course of chronic hypertensive Bright's disease.* We have so far discussed the production of hypertensive renal lesions as a terminal phenomenon in chronic Bright's disease. The malignant termination is by no means constant either clinically or histologically; for example, many cases of chronic nephritis progress to renal failure without developing these manifestations. A similar observation applies to rats with long-sustained hypertension. We have stated that they may die after a 'vascular crisis' and in this case the unclamped kidney may show a combination of acute and chronic lesions, but in the vast majority only chronic lesions are found, such as endarteritis of the arteries and arterioles, varying stages of disorganization and hyalinization in the glomeruli, tubular atrophy and dilatation, and round cell infiltration and fibrosis of the interstitial tissue. We have emphasized by illustrations that it is just this type of chronic lesion, and in particular the glomerular changes, which are found in chronic hypertensive Bright's disease whether this be chronic nephritis, chronic pyelonephritis, malignant hypertension in its later stages, or chronic renal disease following pregnancy toxæmia. It may be objected that such cases frequently do not show the specific arterial lesions in the kidney which we have described in the rat and which occur in malignant hypertension, namely arteriolar necrosis and endarteritis, and that we have therefore no grounds for regarding the changes in the other renal elements as hypertensive in origin. Thus the common arterial changes in these 'non-malignant' cases of hypertensive Bright's disease is elastosis (elastic hyperplasia of the intima) of the larger renal arteries. In answer to this criticism we may say that, firstly, it is possible that in chronic hypertensive states the specific lesions described above may heal or may even result in elastosis. Elastosis occurs in the mesenteric arteries of the rat as the healed stage of lesions resembling periarteritis nodosa (Plate 5, Fig. 12). Furthermore, in rats with chronic hypertension it is often difficult to find more than very occasional examples of endarteritis in the kidneys, although the arteries are obviously abnormal and have the appearance of healed lesions. Secondly, the focal character of the renal damage in the large group of chronic Bright's disease known as chronic interstitial nephritis, is, in view of the similar distribution of the rat lesions, strongly in favour of a vascular origin. We feel justified in concluding, therefore, that not only the mode of termination, but also the progressive course of this group of diseases of the kidney is determined by the production of hypertensive lesions in the renal parenchyma. We do not suggest that

hypertensive renal lesions are the only cause of chronicity in nephritis. A progressive course may obviously result from severe lesions in the acute stage. For example, acute nephritis may run a rapidly progressive course (the 'subacute course' of Volhard) and the histological picture is that of a diffuse glomerulonephritis (with epithelial crescent formation in the glomeruli) which represents a progressive stage of the acute lesion. Similarly, 'subacute parenchymatous' nephritis almost invariably becomes chronic and, until the later stages, hypertension is not usually a conspicuous feature; here again the histological picture is that of a progressive diffuse glomerulonephritis. In both these conditions we frequently encounter a typical malignant termination, but the progressive course is undoubtedly due to a continuation of the primary diffuse inflammation of the kidney. In striking contrast with these disorders there is a large group of cases of chronic nephritis which run a slowly progressive course over many decades without any clinical evidence of recrudescence of the acute form of the disease, yet the condition fails to resolve, the blood-pressure steadily mounts, and when death occurs from renal failure the kidney may present little or no recognizable vestige of the original disease, but only the 'chronic interstitial nephritis' which we have been able to reproduce solely by the effect of hypertension on the kidney. With uncanny clinical insight Volhard (1931) classed these cases as examples of the 'vascular course of chronic nephritis' and our experimental results have simply revealed the mechanism of this vascular course. Thus the condition 'chronic interstitial nephritis' has been so confusing because it is not a disease entity, but a process which is the result of hypertension on the kidney, and as such it may be superimposed on any form of renal disease with persistent high blood-pressure.

These conclusions have an obvious bearing on the classification of Bright's disease. Our work throws no light on the primary cause of either essential hypertension or nephritis, but it provides the reason for the unsatisfactory nature of many of the classifications put forward from time to time both by clinicians and pathologists, namely, that the different types of Bright's disease which in their early stages are clearly distinct, tend as the disease progresses to lose their separate identities, to pursue a common clinical course, characterized by hypertension and renal failure, and to acquire finally a common histological picture in the kidney. Once the view is accepted that the chronic stages of these diseases are chiefly determined by irreversible hypertension, and that this hypertension may arise either outside the kidney or as a result of primary renal damage, then it becomes evident that an acceptable classification can be derived only from the study of the life history of the disease and of primary aetiological factors. As we are still ignorant of the latter we must concentrate on the former, paying special attention to the clinical features in the early stages and correlating these with the histological findings in those cases which die before secondary hypertensive changes have complicated the picture.

In conclusion, reference may be made to an interesting clinical development

of the work on experimental hypertension in animals, the occurrence in man of hypertension due to unilateral renal disease. Several cases have already been reported (Butler, 1937; Leadbetter and Burkland, 1938), where excision of a diseased kidney has led to the disappearance of a previous hypertension. Our investigations on the rat indicate that the possibility of secondary vascular damage in the opposite kidney should not be overlooked, for in this event a residual hypertension might be expected after removal of the diseased kidney; furthermore excision of one kidney might, by increasing the load on a remaining damaged kidney, cause an exacerbation of the hypertension. In cases of longstanding hypertension due to unilateral renal disease we might expect post-mortem examination to reveal one kidney very contracted, with the histological appearances of gross renal damage, for example, in the form of chronic pyelonephritis; the other kidney on the other hand might show hypertrophy and histological changes identical with those of malignant hypertension. Weiss and Parker (1939) have reported such cases and we have observed several examples which will be reported elsewhere.

### *Summary*

1. Experiments are described in which long-sustained hypertension has been produced in rats by partial occlusion of one renal artery, the other kidney being left intact.

2. Chronic lesions are produced in the arteries, glomeruli, tubules, and interstitial tissue of the unclamped kidney which closely resemble those occurring in chronic hypertensive Bright's disease.

3. The character and extent of these lesions are such as might be expected to reduce the blood-flow through the kidney.

4. Removal of the clamped kidney is followed by a residual hypertension in two-thirds of the cases.

5. The degree of residual hypertension is related to the extent of the lesions in the remaining kidney.

6. Acute vascular lesions have been observed in the remaining kidney and in other organs some months after removal of the clamped kidney.

7. These findings support the conception of a vicious circle resulting from the effect of hypertension on the kidney whereby hypertension produces vascular lesions, and these, by reducing the blood-flow through the kidney, aggravate the hypertension; this vicious circle leads to sustained hypertension and progressive renal destruction.

8. The clinical implications of this concept are discussed. In particular the clinical and histological manifestations which are common to the terminal stages of many cases of the different forms of chronic hypertensive Bright's disease are explained.

9. Our findings emphasize the necessity of basing any classification of Bright's disease on the life history and not on the terminal features of the disease.



We should like to express our gratitude to Professor Arthur Ellis whose clinical studies of Bright's disease were the starting-point of these investigations, and to Professor Hubert M. Turnbull for his valuable criticism and for giving us facilities for our work.

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RAT KIDNEY

HUMAN KIDNEY

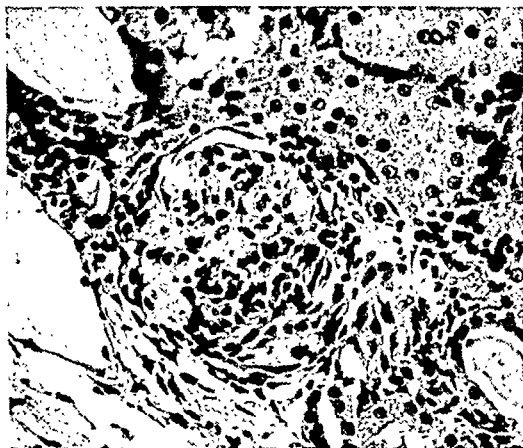


FIG. 3. Rat kidney. Organizing focal necrosis of glomerular tuft with adhesion to Bowman's capsule and almost complete obliteration of capsular space. (Haematoxylin and eosin,  $\times 280$ )

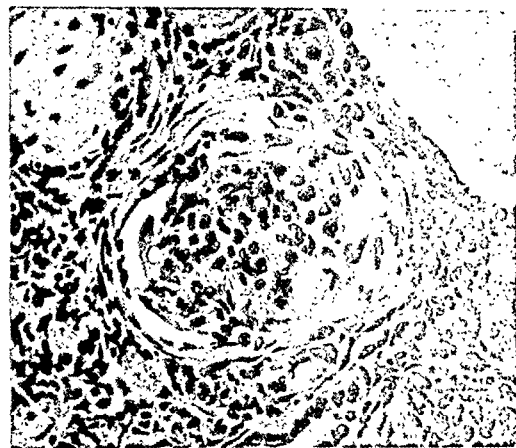


FIG. 4. Human kidney. Case of chronic nephritis. Similar lesion. (Haematoxylin and eosin,  $\times 300$ )

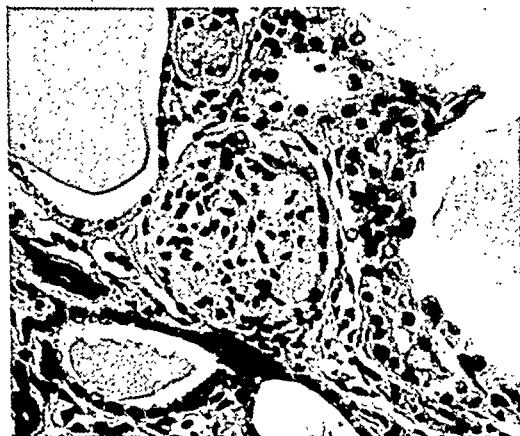


FIG. 5. Rat kidney. Hyaline glomerulus. Loss of capillary outlines; nuclei scattered in pink-staining solid-looking tuft. (Haematoxylin and eosin,  $\times 345$ )

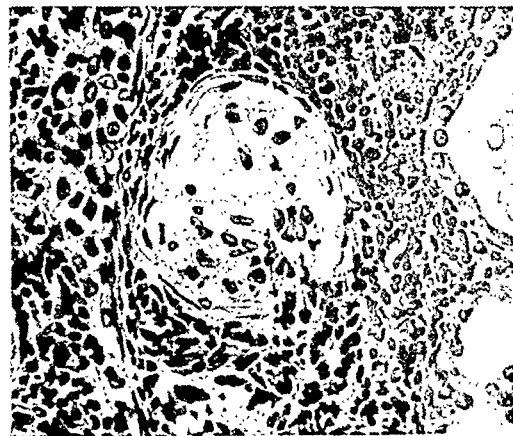


FIG. 6. Human kidney. Case of malignant hypertension. Similar lesion of glomerulus. (Haematoxylin and eosin,  $\times 300$ )

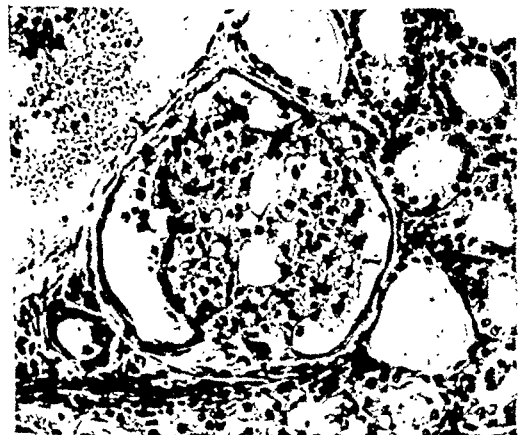


FIG. 7. Rat kidney. Dilatation of capsular space; distension of glomerular tuft with adhesions to Bowman's capsule; swelling and desquamation of capsular epithelium. (Haematoxylin and eosin,  $\times 245$ )



FIG. 8. Human kidney. Case of chronic hypertensive renal disease following toxæmia of pregnancy. Similar lesion of glomerulus. (Haematoxylin and eosin,  $\times 190$ )



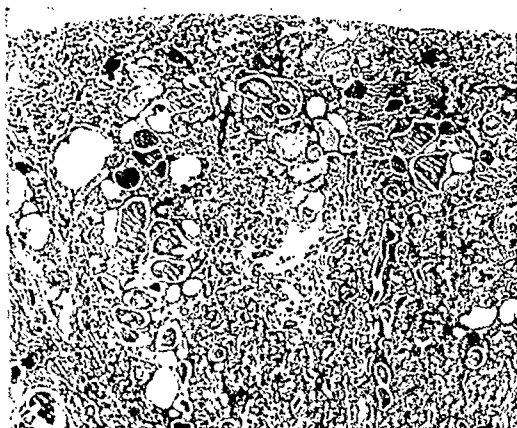


FIG. 9. Rat kidney ('unclamped' kidney). Widespread destruction with wedges of tubular dilatation and interstitial fibrosis. (Haematoxylin and eosin,  $\times 28$ )

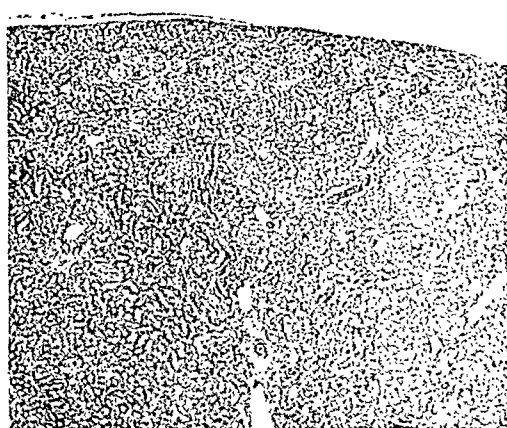


FIG. 10. Rat kidney ('clamped' kidney of same rat). Appears normal. (Haematoxylin and eosin,  $\times 28$ )

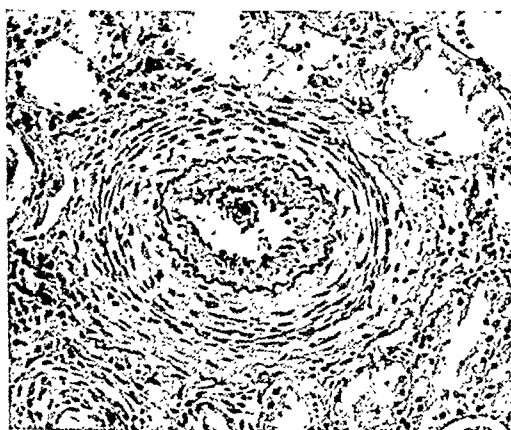


FIG. 11. Rat kidney, cellular thickening of intima (endarteritis) of small artery. (Haematoxylin and eosin,  $\times 205$ )

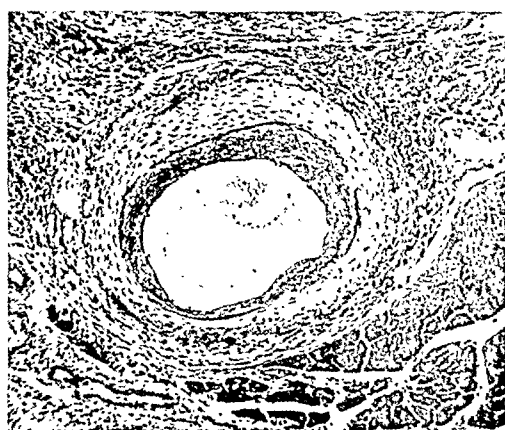


FIG. 12. Rat pancreas. Focal fibrosis of media and elastic hyperplasia in intima of medium sized artery. (Haematoxylin and eosin,  $\times 138$ )

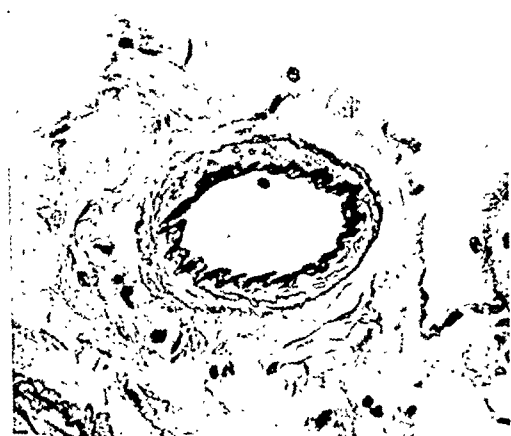


FIG. 13. Rat kidney ('clamped' kidney) small artery showing no medial hypertrophy. (Haematoxylin and eosin,  $\times 470$ )

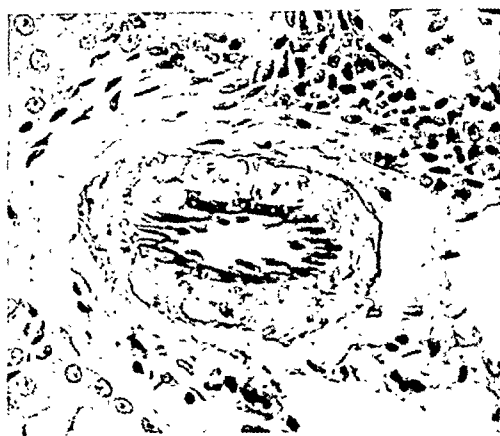


FIG. 14. Rat kidney ('unclamped' kidney of same rat). Small artery showing medial hypertrophy. (Haematoxylin and eosin,  $\times 470$ )



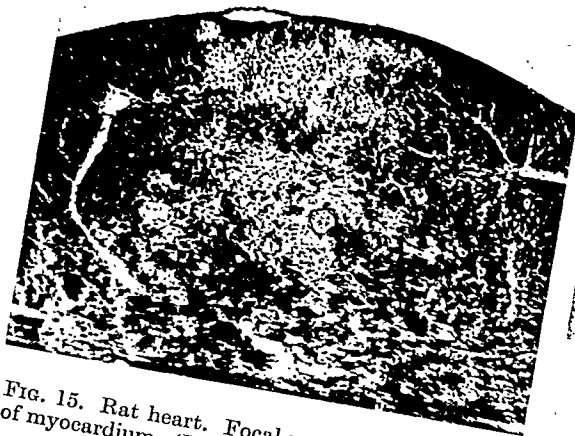


FIG. 15. Rat heart. Focal perivascular necrosis of myocardium. (Haematoxylin and eosin,  $\times 55$ )

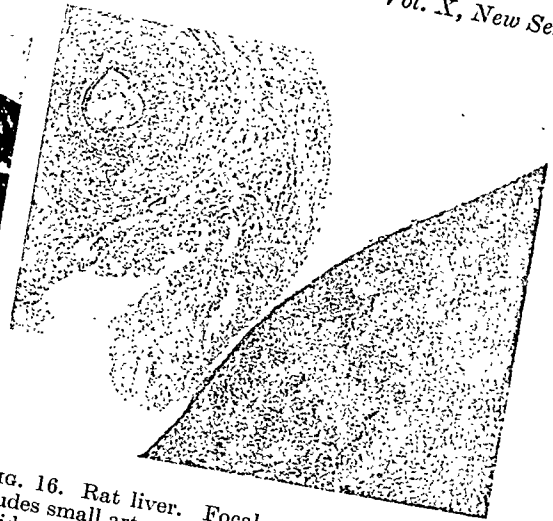


FIG. 16. Rat liver. Focal necrosis; section includes small artery in omentum with acute fibrinoid necrosis and periarteritis. (Haematoxylin and eosin,  $\times 30$ )



FIG. 17. Rat pancreas. Focal necrosis. (Haematoxylin and eosin,  $\times 44$ )



FIG. 18. Rat intestine. Nodes of periarteritis on branches of mesenteric artery. (2/3 natural size)

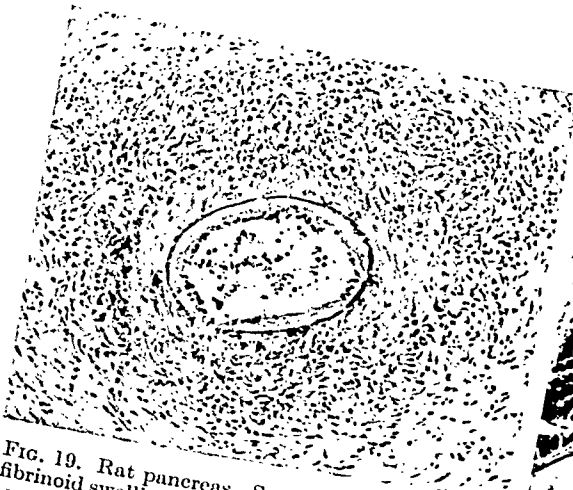


FIG. 19. Rat pancreas. Small artery with acute fibrinoid swelling of intima, necrosis of media and periarteritis. (Haematoxylin and eosin,  $\times 138$ )

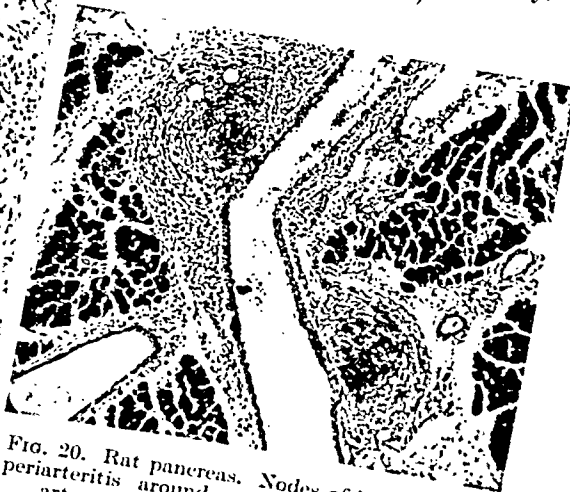


FIG. 20. Rat pancreas. Nodes of haemorrhagic periarteritis around small branches of large artery. (Haematoxylin and eosin,  $\times 64$ )





# METHAEMALBUMIN<sup>1</sup>

## PART I. CLINICAL ASPECTS

By N. HAMILTON FAIRLEY

(From the London School of Hygiene and Tropical Medicine, and the Wellcome Research Institute)

With Plate 7

### *Introduction*

METHAEMALBUMIN (pseudo-methaemoglobin) was first described by Fairley and Bromfield (1934 *a b*) in a patient with blackwater fever seen on the fifth day of the disease. The venous blood was a peculiar chocolate colour and the plasma brown. Investigation showed that the pigment responsible for this peculiar condition was contained in the plasma, but not in the corpuscles, and that while it somewhat resembled methaemoglobin on examination with a Zeiss direct vision spectroscope, differences were demonstrable with the Hartridge reversion spectroscope and in its chemical behaviour with certain reducing and other reagents. Methaemoglobin had previously been described by many observers in blackwater fever plasma, but investigation of a large series of blackwater fever patients and other diseases associated with intravascular haemolysis showed this new pigment and not methaemoglobin to be present. Serial quantitative observations based on the degree of dilution necessary for the extinction of the  $\alpha$  bands of oxyhaemoglobin and this new pigment indicated that extracorporeal oxyhaemoglobin was its ultimate source of origin.

Owing to certain resemblances to methaemoglobin and to the fact that it had been confused with methaemoglobin spectroscopically in plasma from patients with haemoglobinuria and certain haemolytic anaemias, this new pigment was named 'pseudo-methaemoglobin' by Fairley (1937). Some months later, Barkan (1937) and Barkan and Schales (1937) published papers in which the term 'pseudomethaemoglobin' was applied by them to an entirely different pigment. The 'pseudomethaemoglobin' of Barkan probably contains unaltered globin, while the prosthetic group is an oxidized haem or haemin derivative containing trivalent iron with an open porphyrin ring resembling Lemberg's verdo-haemochromogen. The existence of two names, the 'pseudo-methaemoglobin' first described by Fairley and the 'pseudomethaemoglobin' later described by Barkan, for two entirely different compounds, has evidently led to confusion, and later, when Fairley (1938)

<sup>1</sup> Received July 13, 1940.

found that 'pseudo-methaemoglobin' could be synthesized from native serum albumin and alkaline haematin containing trivalent iron, its name was changed to methaemalbumin. In a footnote to their first paper Barkan and Schales stated that while proof-correcting they had just learnt that the title 'pseudo-methaemoglobin' had been already applied by Fairley to another pigment which occurred when haemoglobin or methaemoglobin solutions were incubated in the presence of plasma.

*The Spectroscopic Appearance of Methaemalbumin in Plasma*

In plasma collected from a severe case of blackwater fever some 24 hours after haemoglobinuria has commenced, an  $\alpha$  band in the red is found associated with a diffuse absorption band extending from the short wave-length of the spectrum almost to the D line ( $589\text{ }\mu\mu$ ) when the haemoglobinaemia is intense. When it is less intense the  $\alpha$  band in the red is associated with  $\beta$  and  $\gamma$  bands in the green, the last band merging into or being superimposed on a general absorption. Both these spectroscopic pictures bear a close resemblance to methaemoglobin (Plate 7, Fig. 2) which was first described by Hoppe-Seyler (1865). The spectroscopic similarity of methaemoglobin derived from lysed corpuscles and methaemalbumin in human plasma is shown by the fact that nearly 70 years elapsed before methaemoglobin and methaemalbumin were differentiated by Fairley and Bromfield (1934 *a b*) in blackwater fever and other haemoglobinurias. When examined on the Hartridge reversion spectroscope the  $\alpha$  band of methaemalbumin in the red portion of the spectrum is not collinear with that of methaemoglobin, but is situated at  $623\text{ }\mu\mu$  to  $624\text{ }\mu\mu$ , about midway between methaemoglobin ( $630\text{ }\mu\mu$ ) and sulphaemoglobin ( $618\text{ }\mu\mu$ ), while the well defined  $\beta$  band corresponds to the  $\alpha$  band of oxyhaemoglobin, and the  $\gamma$  band, if visible, is collinear with the  $\beta$  band of oxyhaemoglobin superimposed on a general absorption in the green (Plate 7, Fig. 2). Foy and Kondi (1938) confirmed these findings in an extensive spectrographic analysis of the pigments occurring in cases of blackwater fever in Macedonia.

If one is fortunate enough to obtain a haemoglobin-free specimen of plasma derived from a blackwater fever patient some 24 hours after haemolysis has ceased, or a similar specimen of day blood obtained from a patient with nocturnal haemoglobinuria, the  $\beta$  and  $\gamma$  bands in these situations are absent; the spectrum then consists of an  $\alpha$  band at  $623\text{ }\mu\mu$  to  $624\text{ }\mu\mu$  and a general absorption in the green commencing between  $545\text{ }\mu\mu$  to  $550\text{ }\mu\mu$  and extending towards the short wave-length of the spectrum. Naturally the position of this general absorption band is modified by the concentration of pigments present. Further, owing to this general absorption, it is not as a rule possible with direct spectroscopy to distinguish any other bands due to methaemalbumin in plasma, but this can be done if the pigment be concentrated in the albumin fraction of blackwater fever serum collected some 24 hours after haemoglobinaemia has ceased. In these circumstances, on the Hartridge reversion spectroscope there is the usual well defined  $\alpha$  band

at  $623\ \mu\mu$  to  $624\ \mu\mu$ , a  $\beta$  band at  $540\ \mu\mu$  to  $541\ \mu\mu$ , and a fainter  $\gamma$  band at  $500\ \mu\mu$  to  $501\ \mu\mu$ . These spectroscopic findings correspond closely to those obtained with the reversion spectroscope when methaemalbumin is synthesized by the addition of weakly alkaline haematin (ferric), prepared from pure haemin, to the albumin fraction of normal human serum. The position of these bands as determined on the Hartridge reversion spectroscope differs somewhat from those of Heilmeyer (1933), who investigated by spectrophotometry the absorption curves of alkaline haematin added to blood-serum. He found an absorption curve in the red with a maximum situated at  $620\ \mu\mu$  and an absorption in the green which presented double-headed maxima at approximately  $535\ \mu\mu$  and  $495\ \mu\mu$ . From these and other observations he concluded that alkaline haematin when added to plasma *in vitro* must combine with some protein constituent of the plasma. No spectrophotometric or spectroscopic observations on the plasma from patients with so-called haematinaemia were made by Heilmeyer or other workers on this subject; in consequence, the possible clinical significance of these *in vitro* experiments with haematin and serum appears to have been overlooked.

*The Differentiation of Methaemalbumin from Methaemoglobin and Sulphaemoglobin*

As the  $\alpha$  band of methaemalbumin lies at  $623\ \mu\mu$  to  $624\ \mu\mu$ , approximately midway between that of methaemoglobin at  $630\ \mu\mu$  and of sulphaemoglobin at  $618\ \mu\mu$ , considerable difficulty may be experienced by the clinical pathologist in differentiating these three pigments spectroscopically if a reversion spectroscope be not available.

*Distribution of the pigment in blood.* Observations during the past six years have shown that the distribution of these pigments is very constant. In methaemalbuminaemia if oxalated blood be centrifuged, the plasma and corpuscles separated, and the corpuscles subsequently washed and lysed in distilled water, methaemalbumin is demonstrable in the brown plasma, while the lysed corpuscles show only oxyhaemoglobin. When methaemoglobin or sulphaemoglobin appears in the blood, as, for example, in sulphanilamide and other drug poisoning, both these pigments are found exclusively within the dark brown corpuscles and not free in the plasma. The intracorpuseular location of methaemoglobin and sulphaemoglobin, and the constant extracorpuseular distribution of methaemalbumin in the plasma constitute readily demonstrable differences. In haemoglobinuria the urine contains oxyhaemoglobin and, if it be acid in reaction, generally methaemoglobin as well, but not sulphaemoglobin or methaemalbumin. Methaemoglobinuria is always secondary and never primary, the methaemoglobin being derived from the oxyhaemoglobin as it passes down the renal tubules.

*Chemical differentiation.* The most rapid method of differentiating the three pigments is by studying the effect of different chemical reagents on their  $\alpha$  bands. For this purpose a direct vision spectroscope is all that is required.

*Stokes's reagent.* If 2 drops of freshly prepared Stokes's reagent be added per 1 c.c., the  $\alpha$  band of methaemoglobin is immediately dispersed; with methaemalbumin and sulphaemoglobin it persists unaltered.

*Dilute ammonium sulphide (10 per cent.).* The addition of 1 drop per 1 c.c. of 10 per cent. ammonium sulphide immediately disperses the  $\alpha$  band of methaemoglobin, while that of methaemalbumin and sulphaemoglobin persists unaltered.

*Concentrated ammonium sulphide.* When concentrated ammonium sulphide is added in the proportion of 0.1 c.c. to each 1 c.c. of plasma, the  $\alpha$  band is dispersed with both methaemoglobin and methaemalbumin. In the presence of methaemoglobin reduced haemoglobin is produced; with methaemalbumin a haemochromogen is formed with its  $\alpha$  band situated at  $558 \mu\mu$ . The  $\alpha$  band of sulphaemoglobin remains unaltered.

There are numbers of other differences such as the effect of changing the pH by the addition of alkali, the action of sodium hydrosulphite, potassium cyanide (1 per cent.), hydrogen peroxide (10 volumes), and hydrazine hydrate (10 per cent.), but the simple procedures outlined above suffice to differentiate clearly these three pigments in specimens of oxalated blood sent to the clinical pathologist.

*Mixtures of methaemalbumin and methaemoglobin in plasma.* When a solution of methaemoglobin was added to methaemalbumin in plasma, it was found that the  $\alpha$  absorption band lay somewhere between  $623 \mu\mu$  and  $630 \mu\mu$  according to the relative concentrations of the pigments present. When the pH was changed to 9.0 by the addition of N/1 sodium hydroxide (1 drop per 2 c.c.) the neutral methaemoglobin was converted into alkaline methaemoglobin, leaving the  $\alpha$  band of methaemalbumin clearly defined at  $623 \mu\mu$  to  $624 \mu\mu$ . The addition of 10 per cent. ammonium sulphide produced a similar result, for it disperses the  $\alpha$  band of methaemoglobin, leaving that of methaemalbumin intact at  $623 \mu\mu$  to  $624 \mu\mu$ . Though theoretically possible, the writer has never encountered methaemalbumin and methaemoglobin occurring together in human plasma in disease; methaemoglobin or sulphaemoglobin may be present within the corpuscles following sulphanilamide treatment in diseases associated with methaemalbuminaemia.

#### *Other Chemical and Physical Reactions of Methaemalbumin*

*Methaemalbumin in plasma.* An initial difficulty experienced in studying the chemistry of methaemalbumin in human plasma was to obtain samples free from haemoglobin. This obviously is impossible in any case of haemoglobinuria while intravascular haemolysis is still proceeding. Twenty-four hours after haemoglobinuria has stopped, the haemoglobinaemia will have disappeared, but methaemalbuminaemia persists. Any haemoglobin now present is due to haemolysis, but if thick layers of plasma be examined spectroscopically it is surprising to find how difficult it is, even with the most careful technique, to collect samples entirely free from haemoglobin. When selected samples of plasma containing methaemalbumin, but only

minute traces of oxyhaemoglobin, have been reduced with sodium hydrosulphite ( $\text{Na}_2\text{S}_2\text{O}_4$ ) a reddish colour is produced and a two-banded, not a broad single-banded, spectrum immediately appears with an  $\alpha$  band at approximately  $573\mu\mu$  and a  $\beta$  band at approximately  $528\mu\mu$ . If carbon monoxide be now bubbled through plasma containing methaemalbumin treated with sodium hydrosulphite a cherry-red solution results with the production of a two-banded spectrum very similar to that of carboxyhaemoglobin. Similarly, if 10 per cent. sodium hydroxide be added to serum containing methaemalbumin treated with sodium hydrosulphite a haemochromogen is formed indistinguishable on the Hartridge reversion spectro-scope from globo-proto-haemochromogen. In order to study the chemistry of methaemalbumin under better conditions and to eliminate as far as possible reactions due to traces of oxyhaemoglobin, it was decided to concentrate the pigment in the albumin fraction of human serum.

*Methaemalbumin in the albumin fraction of human serum.* In the haemoglobinurias and in haemolytic anaemia with methaemalbuminaemia it always proved possible to concentrate this pigment in the albumin fraction of the serum. The following findings were typical.

A specimen of brown serum derived from a fatal case of blackwater fever, sent by Dr. Henry Foy from Salonika, was investigated from this viewpoint. On arrival it contained no demonstrable oxyhaemoglobin. The  $\alpha$  band was situated at  $623\mu\mu$  and the  $\beta$  band at  $540\mu\mu$ ; the  $\gamma$  band was obscured in the general absorption. It gave the typical reactions differentiating methaemalbumin from sulphaemoglobin and methaemoglobin, and Schumm's test was positive. An equal volume of saturated ammonium sulphate solution was added and the precipitated globulin filtered off. The precipitate contained only a trace of methaemalbumin as indicated by a weak positive Schumm's test. The brown filtrate containing the albumin fraction was then saturated with ammonium sulphate; the filtrate was water-clear and the precipitate brown. The latter was dissolved in distilled water and again precipitated with ammonium sulphate; subsequently this process was repeated twice. Finally the precipitate was dissolved in distilled water, dialysed in 'viscose' sacs in running water for several days, and finally in distilled water until free from all traces of ammonium sulphate. Examination on the reversion spectro-scope showed an  $\alpha$  band at  $624\mu\mu$ , a  $\beta$  band at  $540\mu\mu$ , and a  $\gamma$  band at  $501\mu\mu$ .

*Haemalbumin.* On reduction with sodium hydrosulphite ( $\text{Na}_2\text{S}_2\text{O}_4$ ) the colour changed from brown to red and a two-banded spectrum with a prominent  $\alpha$  band at  $572\mu\mu$  and a  $\beta$  band at  $531\mu\mu$  was immediately formed, due presumably to the formation of a new compound, haemalbumin, containing ferrous iron.

*Carboxyhaemalbumin.* On bubbling carbon monoxide through methaemalbumin reduced with sodium hydrosulphite a cherry-red solution resulted. Spectroscopic examination showed a two-banded spectrum very similar to that of carboxyhaemoglobin, the  $\alpha$  band being situated at  $570\mu\mu$  to  $571\mu\mu$  and the  $\beta$  band at  $533\mu\mu$  to  $535\mu\mu$ .

*Albumin-fraction haemochromogen.* On treating methaemalbumin contained in the albumin fraction with sodium hydrosulphite and sodium hydroxide (10 per cent.) a haemochromogen was formed which was indistinguishable

from globo-proto-haemochromogen. Hewitt (1936, 1937, 1938) has shown that the albumin fraction contains several proteins including crystalalbumin, globoglycoid, and seroglycoid; all of these would be denatured by alkali and combine with reduced haematin (ferrous) to form haemochromogens. It follows that the compound responsible for the  $\alpha$  band at  $558\mu$  cannot be attributed exclusively to albumin-haemochromogen.

*Behaviour of plasma methaemalbumin in the ultracentrifuge.* Dr. A. S. McFarlane investigated the behaviour of methaemalbumin in serum collected from a fatal case of blackwater fever, the patient dying from uraemia on the seventh day from onset of the haemoglobinuria. The plasma was dark brown in colour and contained high concentrations of methaemalbumin and bilirubin (19 units). In the blood, bilirubin is present in combination with albumin as shown by the ultracentrifuge studies of Pedersen and Waldenström (1937), and as considerable quantities were present in combination with albumin in this specimen it became necessary for Dr. McFarlane to devise special optical conditions to differentiate the two pigments. When this had been done it was found that the brown pigment, methaemalbumin, sedimented at the same rate as serum albumin.

*Comment.* Though the spectroscopic picture and chemical reactions of methaemalbumin in plasma or concentrated in the albumin fraction of human serum are not in all respects analogous to methaemoglobin, its synthesis from alkaline haematin (ferric) and serum albumin and its general behaviour indicate that in methaemalbumin the prosthetic group consists of oxidized haematin containing trivalent or ferric iron, while the protein component is native serum albumin instead of native globin. The failure to combine loosely with oxygen when in the ferrous state may be due either to absence of polymerization such as occurs in haemoglobin and heliocorubin, or to its different protein components.

#### *Schumm's Haemochromogen Test*

When concentrated ammonium sulphide is added to an aqueous solution of alkaline haematin (ferric), ammonium-haemochromogen is formed, provided the commercial preparation used contains enough ammonia for the purpose. This test, introduced by Schumm (1912), has been widely used in clinical medicine to demonstrate haematin in plasma. A positive reaction is characterized by the appearance of a haemochromogen with a sharply defined  $\alpha$  band at  $558\mu$ . Van den Bergh and Snapper (1915) emphasized the difficulty of doing the test in the presence of much oxyhaemoglobin, since reduced haemoglobin is formed which obscures the haemochromogen bands. Dilution of the plasma to lessen the concentration of haemoglobin dilutes the pigment responsible for the reaction to such a degree that the  $\alpha$  band of haemochromogen is no longer visible unless present in considerable concentration. The method now adopted in performing the test is to cover a given volume of serum with a layer of ether and run in with a pipette one-tenth the volume of concentrated ammonium sulphide, subsequently mixing the solutions by shaking. In our work concentrated ammonium sulphide

(Analar) was employed, but it was found that unless a few drops of ammonia or slightly more than one-tenth the volume of ammonium sulphide was added, a haemochromogen was not generally demonstrable with dilute aqueous solutions of alkaline haematin (ferric). If, however, to the same amount of alkaline haematin mammalian serum were added, the test proved invariably positive. This was due to serum proteins being denatured and a serum-haemochromogen being formed.

Schumm's haemochromogen test was invariably positive with human serum or plasma in which methaemalbumin could be demonstrated spectroscopically, and since haematin does not remain free in plasma, but always combines with serum albumin to form this pigment, it follows that a positive Schumm's test in human plasma indicates methaemalbumin. When blackwater fever plasma was diluted until methaemalbumin was no longer visible spectroscopically in a layer as thick as 10 cm., the haemochromogen test was still positive. It follows that the haemochromogen test for methaemalbumin affords a much more sensitive means of detecting this pigment in plasma than direct spectroscopic examination. Subsequent investigation showed it to be immaterial whether the methaemalbumin had been synthesized by adding alkaline haematin (ferric) to human serum-albumin fraction or to crystalalbumin, separated in the albumin fraction from the plasma of a patient with methaemalbuminaemia, or was present in blackwater fever plasma. When concentrated ammonium sulphide is added to methaemalbumin synthesized from haematin and crystalalbumin, the ferric haematin is reduced to ferrous haematin and the albumin moiety is denatured with the production of an almost pure serum-albumin-haemochromogen. When examined on the Hartridge reversion spectroscope the  $\alpha$  band at  $558\ \mu\mu$  is collinear with the haemochromogen formed when alkaline haematin is added to recrystallized horse albumin solution or human globulin solution and subsequently treated with concentrated ammonium sulphide; it differs from the ammonium-haemochromogen formed by the addition of an excess of concentrated ammonium sulphide to an aqueous solution of alkaline haematin. The addition of concentrated ammonium sulphide to plasma containing methaemalbumin certainly results in the formation of serum-albumin-haemochromogen, but if the haematin (ferric) is split off from the serum albumin as well as being reduced to ferrous haematin, some of the latter may unite with other denatured proteins in the serum to form globulin-haemochromogen, seroglycoid-haemochromogen, and globoglycoid-haemochromogen. Perhaps in the present state of knowledge, the general term sero-haemochromogen would best describe the end product of the reaction, for it would include all the denatured serum-proteins which might combine with reduced haematin (ferrous) to form haemochromogens.

#### *Classification of Anaemias Presenting Abnormality in the Blood Pigments*

A considerable amount of literature exists on haematinaemia in man based on Schumm's test. Haematinaemia has been described clinically by

many workers including Schumm (1912), van den Bergh and Snapper (1915), Bingold (1932), and Duesberg (1933) in a variety of conditions, including pernicious anaemia, lead poisoning, gas gangrene infections, malarial fever, certain chemical poisonings, and acute yellow atrophy of the liver. By none of these observers did the spectroscopic appearances of haematin in the circulating blood receive special consideration, though the presence of such pigments as methaemoglobin and sulphaemoglobin with an  $\alpha$  band in the red portion of the spectrum was reported. Van den Bergh and Engelkes (1922) proposed the term *parhaemoglobinaemia* for those clinical conditions in which methaemoglobin and sulphaemoglobin were found in the blood and recognized four types—*intraglobular methaemoglobinaemia without haemolysis*, *intraglobular sulphaemoglobinaemia without haemolysis*, *haemolytic methaemoglobinaemia*, and *haemolytic sulphaemoglobinaemia*.

Bingold (1932) suggested classifying the cases of haemolytic anaemia and haemolytic icterus into three groups according to the different blood pigments present—bilirubin without haematin, bilirubin with haematin, and oxyhaemoglobin, bilirubin, haematin, and methaemoglobin. The present work indicates that haematin cannot exist as such in the circulating blood, for when formed it combines with serum albumin to form methaemalbumin, which is responsible for a positive Schumm's test in plasma; if present in sufficient concentration methaemalbumin gives a distinctive spectroscopic picture which in the past has been erroneously recorded as methaemoglobin or sulphaemoglobin. In the writer's opinion both the haemolytic methaemoglobinaemia and the haemolytic sulphaemoglobinaemia of van den Bergh were examples of methaemalbuminaemia. In enterogenous cyanosis and drug poisonings both these pigments occur only within the corpuscle. Intra-corpuscular methaemoglobin is readily converted back to oxyhaemoglobin in the body, and in drug poisoning soon disappears provided administration of the drug be stopped. Sulphaemoglobin on the other hand is an irreversible ferrous derivative of haemoglobin and some oxidation product of sulphuretted hydrogen according to Keilin (1933); once formed it probably persists throughout the life of the corpuscle. There is no evidence that primary sulphaemoglobinuria or methaemoglobinuria ever occurs. Should there be any lysis of corpuscles containing either methaemoglobin or sulphaemoglobin, the presence of these pigments in the circulating blood would be transient and would probably not attain a sufficient concentration to be visible spectroscopically, since both are spectroscopically insensitive and have to be present in considerable amounts to be demonstrated. On incubating these pigments in the presence of plasma at 40°C., methaemalbumin is formed in 24 to 48 hours, and it appears probable that a similar katabolic route would be followed in the circulating blood. In view of the new data now available it is evident that both van den Bergh's and Bingold's classifications have outlived their usefulness.

The haemolytic anaemias studied in the present series fall naturally into three groups according as they show :



- (I) Haemoglobinuria, methaemalbuminaemia, and hyperbilirubinaemia.
- (II) Methaemalbuminaemia and hyperbilirubinaemia.
- (III) Hyperbilirubinaemia alone.

This classification has the additional advantage of indicating the predominant or exclusive site of haemolysis which in Groups I and II is in the bloodstream, and in Group III within the reticulo-endothelial system.

*Group I. Haemolytic anaemia associated with haemoglobinuria, methaemalbuminaemia, and hyperbilirubinaemia.* This group includes all the well recognized haemoglobinurias except myohaemoglobinuria. Owing to the low renal threshold for myohaemoglobin and its rapid excretion by the kidneys, there is probably not time for the formation of methaemalbumin in the circulating blood. This is suggested as a result of our observations on a single case of myohaemoglobinuria, specimens from which were sent by Dr. D. Hunter.

*Blackwater Fever.* Much of the work done on methaemalbumin has centred around the study of blackwater fever plasma. Methaemoglobin was recorded in the plasma of blackwater fever patients by Arkwright and Lepper (1918), Yorke, Murgatroyd, and Owen (1930), Ross (1932), and Fairley and Bromfield (1934*a*). In the same year Fairley and Bromfield (1934*b*) recorded the fact that the pigment formerly regarded as methaemoglobin in the plasma of blackwater fever patients was a new pigment. After the lapse of a year this pigment was encountered in another case of blackwater fever in London and again proved not to be methaemoglobin. Subsequent investigation in Macedonia by the same observers (1937) showed that this pigment was almost invariably present in the plasma, but not in the urine, whereas methaemoglobin was present in the urine, but never demonstrable in the plasma. For reasons already discussed, this pigment was first named pseudo-methaemoglobin and later methaemalbumin. These findings were confirmed by Foy and Kondi (1938) who made a spectrophotographic analysis of the pigments in the serum and urine of blackwater fever cases. From a large number of spectrograms taken, the centre of the  $\alpha$  band of this pigment was found to be subject to a certain amount of variation ranging within  $622\ \mu\mu$  and  $624\ \mu\mu$ . It was thought that this instability might be due to variation in pH as had been found in the case of methaemoglobin by Heilmeyer (1933).

In the present series of 10 cases of blackwater fever, serial biochemical observations were made at different stages of the disease. As a routine, investigations included examination of plasma with the reversion spectroscope, the use of Harrison's apparatus which permits a depth of fluid from 0.2 to 12 cm. to be examined spectroscopically, Schumm's ammonium sulphide test, and various chemical tests referred to previously. The results obtained are incorporated in the Table, from which it will be seen that methaemalbumin was present in the plasma at some stage in all 10 cases of Series II, being demonstrable in a depth of from 0.5 to 4.0 cm. Schumm's test was not carried out in the first series, but was invariably positive in the second. Had cases in Series I been examined by a similar technique it is

probable that the plasma from both cases, in which only haemoglobin was demonstrated, would have been found to contain methaemalbumin at some time during the course of the illness. In both series there was a time lag in the appearance of methaemalbumin which was not demonstrable during the first few hours after the onset of haemoglobinuria. Serial quantitative observations based on the degree of dilution necessary for the extinction of the  $\alpha$  bands of oxyhaemoglobin and methaemalbumin suggested that extracorpuseular oxyhaemoglobin was its ultimate source, since methaemalbumin appeared later and reached its maximum concentration 16 to 35 hours from

*Blood-pigments in the Plasma of Blackwater Fever Patients*

Macedonian and London Series	Total cases	Oxyhaemoglobin alone	Oxyhaemoglobin and methaemalbumin	Methaemalbumin alone	Bilirubin (van den Bergh units)
I	14	2	10	2*	7.0 to 88.5
II	10	0	10	0	3.5 to 95.0
Total	24	2	20	2	

\* First seen after haemoglobinuria had ceased.

onset of the haemoglobinuria, by which time, especially in fatal cases, the haemoglobinaemia had often decreased or actually disappeared.

In mild cases, where the haemoglobinaemia and haemoglobinuria were of short duration, methaemalbumin was occasionally no longer demonstrable spectroscopically on the second day, though Schumm's test might still be positive. In severe cases methaemalbumin often persisted for a period of 4, 5, or 6 days, and in one desperately ill patient, where there was recurrent haemolysis, it was demonstrated up to the 14th day from onset of haemoglobinuria. In patients, where methaemalbumin persisted in the plasma over these longer periods of time, recurrent intravascular haemolysis with or without haemoglobinuria was almost certainly occurring. Another factor calls for consideration in such circumstances, namely, the rate of excretion or destruction of the pigment in the body. Duesberg (1933) injected haematin intravenously in man and found that no increase in plasma bilirubin resulted. In repeating Duesberg's experiment I injected alkaline haematin (ferric) in a dosage of 4 mg. per kilo. No increase in bilirubin was observed, but methaemalbumin and not haematin was demonstrable spectroscopically by Harrison's apparatus for a period of approximately 30 hours, while the plasma yielded a positive Schumm's test for 45 hours or longer.

Rimington (1939) estimated the porphyrin excretion on one of these patients after injections of alkaline haematin (ferric) and found an increase in faecal porphyrin and a less marked secondary increase in the urinary porphyrin level; he concluded from these and other observations on monkeys and rabbits that there is an alternative route of haemoglobin breakdown by which the oxidized (ferric) or protein-free pigment is ultimately converted, not to bile pigment, but to porphyrin. Available evidence suggests that in intravascular haemolysis of any magnitude katabolism of some of the

circulating haemoglobin results in the production of oxidized haematin (ferric), which combines with serum albumin to form methaemalbumin. This is subsequently broken down by the liver, the pigment moiety being excreted as Series III porphyrin in the faeces.

On injecting haemoglobin (5 gm.) intravenously in man Duesberg (1933) produced haemoglobinaemia and hyperbilirubinaemia, but he found Schumm's test was invariably negative unless there was hepatic disease. Recently, using larger quantities of haemoglobin (14 to 25 gm.), I have repeated these observations and have invariably found, even in normal subjects with an alkalized urine, that the plasma developed a positive Schumm's test, generally within 5 hours, and continued to give a positive reaction for 28 to 48 hours or longer, but the concentration of methaemalbumin was never sufficient to enable the pigment to be demonstrated spectroscopically. In two patients, who were transfused with incompatible blood and received inadvertently larger quantities of haemoglobin (approximately 45 and 90 gm.), methaemalbumin was demonstrated in the plasma in both instances. Renal involvement led to anuria with fatal consequences in the latter case. In fatal cases of blackwater fever, as well as in incompatible transfusion, parenchymatous degeneration of the liver cells has often been reported; in such circumstances, it would not be remarkable if the katabolism of methaemalbumin by the liver was retarded sufficiently to lead to its retention for a longer period in the circulating blood. Clinically, there is evidence favouring this view, for where intravascular haemolysis co-exists with liver disease the methaemalbuminaemia is most marked.

*Nocturnal haemoglobinuria (Machiafava-Micheli syndrome).* In nocturnal haemoglobinuria both extracorpuscular haemoglobin and methaemoglobin have been recorded in plasma collected during the haemoglobinuric phase by several observers, and Scott, Robb-Smith, and Scowen (1938) recently stressed the importance of examining the plasma for methaemoglobin in all cases of acquired haemolytic anaemia. Fairley and Bromfield (1939), at a laboratory meeting of the Royal Society of Tropical Medicine and Hygiene, demonstrated that the pigment with an  $\alpha$  band in the red portion of the spectrum in the plasma obtained from a case of nocturnal haemoglobinuria, was methaemalbumin (pseudo-methaemoglobin) and not methaemoglobin. It gave the typical spectrum and chemical reactions of the former pigment. Since then these findings have been confirmed in the plasma from two further cases, one sent by Dr. J. C. Young and the other by Dr. van den Berghe. A significant haemoglobinaemia was found in samples of the night blood, and provided the plasma was examined through a sufficient depth (2 to 6 cm.), methaemalbumin was demonstrable spectroscopically in specimens of both day and night blood. Schumm's haemochromogen test was invariably positive in all specimens tested, while the indirect van den Bergh reaction was positive in both cases examined, though in one it did not exceed 1.5 units.

*Anaerobic sepsis.* Anaerobic sepsis was included by van den Bergh (1922) in his classification as a cause of both haemolytic methaemoglobinaemia and

haemolytic sulphaemoglobinaemia, while intense haematinaemia has been reported in infections with *Clostridium welchii*. Hill (1936), in his series of post-abortal and puerperal gas gangrene, stated that the blood-serum in every case was burgundy-coloured, and biochemical and spectroscopic examination revealed the presence of free oxyhaemoglobin and methaemoglobin. In seven cases the urine was port-wine-coloured and contained oxyhaemoglobin and methaemoglobin. In view of the universal recognition of methaemoglobinaemia and haematinaemia in anaerobic sepsis the following case of *Clostridium welchii* infection with haemolytic anaemia and probable haemoglobinuria is of interest.

The patient, a man aged 62 years, had been operated on for herniorrhaphy and fascia lata graft. Postoperative soiling of the wound with faeces occurred (23/7/39), and infection followed with a seropurulent discharge, fever, and symptoms of toxæmia. It was noted that the urine became port-wine-coloured, but it was not investigated at the time for blood-pigments. 23/7/39. Anaerobic culture of the pus showed *Clostridium welchii*, and non-haemolytic streptococci were also cultured. Several blood transfusions were given and sulphanilamide therapy instituted. 29/7/39. Red cells were 2,960,000 per c.mm., haemoglobin 60 per cent., and colour index, 1.0. Five days later the red cells were 2,690,000 per c.mm., haemoglobin 50 per cent., and leucocytes 15,000 per c.mm. 1/8/39. Methaemalbumin was demonstrated spectroscopically in a layer of plasma 6 cm. thick, the  $\alpha$  band not being dispersed by Stokes's reagent or 10 per cent. ammonium sulphide. Schumm's test was positive. Van den Bergh test, direct reaction negative, indirect positive (1.5 units). The corpuscles were washed free of plasma and laked with distilled water. An  $\alpha$  band at  $618\mu\mu$  was observed spectroscopically; this was not discharged with sodium hydrosulphite, and was therefore due to sulphaemoglobin. The urine was dark brown in colour and contained albumin and urobilin, but no bile or blood-pigment.

The finding of sulphaemoglobin in the corpuscles associated with methaemalbumin in the plasma was of considerable interest, as it was the first occasion on which the two pigments had been encountered together in blood. Subsequent inquiries, however, elicited the fact that the patient had received sulphanilamide therapy which was no doubt responsible for the associated sulphaemoglobinaemia. *Clostridium welchii* is well known to produce a haemolytic anaemia with or without haemoglobinuria, and the history of 'port-wine' urine suggests that there was a haemoglobinuric phase possibly controlled by early sulphanilamide therapy. At the time the urine was biochemically investigated there was a significant urobilinuria, but no haemoglobinuria.

*Haemolytic anaemia associated with haemoglobinuria and splenomegaly.* During the course of the present investigation an opportunity arose of studying a number of obscure haemolytic anaemias associated with splenomegaly with or without haemoglobinuria. Biochemical data indicated that corpuscles were being destroyed in the blood-stream. Whether such red-cell destruction is due to an actual lysis, or whether it results from the production by the red marrow of an abnormal type of corpuscle especially prone to haemolysis,

is unknown. A marked hyperplastic reaction of the red-cell series, generally normoblastic in type, is evident in smears obtained by sternal puncture, while the percentage of reticulocytes and nucleated red cells observed in the blood smears indicates the pressure under which the erythroblastic tissue is working in its efforts to replace the destroyed corpuscles.

*Case I.* An Indian doctor, aged 49 years, was admitted to St. Mary Abbots Hospital in May 1939, with a history of anaemia and recurrent bouts of haemoglobinuria, which on occasion appeared to be precipitated by liver extract therapy in much the same manner as blackwater fever by quinine treatment. Attacks of haemoglobinuria, however, occurred independently of this treatment. There had been no malarial fever for some years.

Physical examination showed a somewhat jaundiced patient with pale mucous membranes, an enlarged hard spleen (Schüffner 2) and a firm liver, the edge of which was palpable three fingers' breadth below the costal margin in the nipple line.

Laboratory Investigations. Red cells, 2,370,000 per c.mm., haemoglobin 50 per cent., colour index 1.1, leucocytes 5,100 per c.mm.; neutrophils 64 per cent., eosinophils 2.5 per cent., lymphocytes 27.5 per cent., monocytes 6 per cent. Marked anisocytosis and polychromasia. Nucleated red cells 51 per c.mm. The plasma was dark yellowish-brown in colour. Oxyhaemoglobin demonstrable in layer of 0.2 cm. thickness, indicating significant oxyhaemoglobinaemia. Methaemalbumin, visible spectroscopically in depth of 1.0 cm. of plasma. Other typical chemical reactions for methaemalbumin given. Schumm's test was strongly positive. Van den Bergh, direct negative, indirect positive (9.0 units).

Urine, dark brown in colour, specific gravity 1010, reaction acid, albumin ++, urobilin ++, porphyrins present, oxyhaemoglobin and methaemoglobin both present, occasional epithelial cells, very few red blood-cells and granular casts.

Progress. The patient left hospital in September at the outbreak of war with a very large liver and a palpable spleen, and in January 1940 wrote saying he had obtained a post as a ship's surgeon and was feeling well.

*Case II.* Specimens of blood from this case were sent by Dr. J. F. Wilkinson from the Royal Infirmary, Manchester. The patient, a man aged 31 years, a card-room operator, was first seen in June 1938. He had had dyspnoea, anaemia, splenomegaly, and gradually increasing jaundice of three years' duration. Since April 1936 haemoglobinuria had been noted from time to time; the blood-pigment appeared usually at night, and it was always precipitated by blood transfusion. Dr. Wilkinson reported that in June 1938 the haematological findings were as follows: red cells 990,000 per c.mm., haemoglobin 21 per cent., colour index 1.05, leucocytes 6,400 per c.mm.; differential count normal, slight anisocytosis and poikilocytosis, polychromasia marked, no nucleated red cells, reticulocytes numerous (20 to 40 per cent.), platelets scanty. Van den Bergh test, indirect positive (10 units). Sternal marrow at biopsy showed severe hyperplastic reaction of normoblastic type. The Wassermann reaction, red-cell fragility, and coagulation time were normal.

On 27/6/38 the spleen, which was greatly enlarged, was removed without difficulty, and a rapid increase in the platelets followed. On histological examination, the spleen showed little disturbance of its normal architecture. The malpighian bodies were of normal size, though some had hyperplastic

centres. The sinuses in the pulp were well defined, but showed no hypertrophy of their walls. The pulp contained red cells and white cells in normal relation, though there was some reduction in polymorphonuclears. There was no suggestion of the overfilling with blood characteristic of acholuric jaundice, or of the histiocytic proliferation seen in certain other types of haemolytic anaemia. The picture might be described as a non-specific change in the direction of increased cellularity of the pulp, rather similar to that seen in certain purpuras.

Progress. The anaemia at times had been megalocytic with a high colour index; more often the colour index varied between 0.7 and 1.1, and the reticulocytes from 20 to 40 per cent. Since splenectomy he had experienced variable but reasonable health. 29/3/39. Red cells 1,860,000 per c.mm., haemoglobin 49 per cent., colour index 1.3, reticulocytes 23.5 per cent. Several transfusions were given and four weeks later the red cells were 2,330,000 per c.mm., haemoglobin 46 per cent., and colour index 1.0. 29/4/39. Plasma was brownish-yellow and showed a trace of oxyhaemoglobin, while methaemalbumin was visible spectroscopically in a thickness of 2.5 cm. Schumm's test was strongly positive. 28/8/39. He commenced coughing up bloodstained mucus and died the same day.

In both these cases hyperbilirubinaemia, methaemalbuminaemia, haemoglobinaemia, and haemoglobinuria co-existed with evidences of intense blood regeneration. In Case I a greater concentration of methaemalbumin was present in the plasma than would have been anticipated from the degree of blood destruction. The liver was firm and enlarged from hepatic congestion and possibly cirrhosis. In this instance the high concentration of methaemalbumin may have been partly due to defective transformation of methaemalbumin into faecal porphyrin by the liver. The possible role of malaria in the aetiology of such cases as this will be considered later.

*Group II. Haemolytic anaemia with methaemalbuminaemia but no haemoglobinuria.* In this group there is hyperbilirubinaemia and the plasma contains methaemalbumin which is demonstrable spectroscopically in the more severe cases or, when intravascular destruction of blood is less marked, by a positive Schumm's test. Clinical evidence of haemoglobinuria is absent, since the concentration of haemoglobin in the circulating blood is either not sufficient to exceed the renal threshold, or if haemoglobin reaches the tubules, it is present in the urine in amounts too small to be demonstrated by methods ordinarily employed in the clinical laboratory.

*Haemolytic anaemia with splenomegaly of unknown cause.* As already suggested, the difference between this form of haemolytic anaemia and the type described in the preceding section associated with haemoglobinuria is one of degree rather than of kind, and between the bouts of haemoglobinuria no biochemical differences are demonstrable. A low-grade haemoglobinaemia may exist without haemoglobinuria in both types, but in diagnosing haemoglobinaemia it is necessary to remember that a definite standard must be adopted, for despite careful technique, some degree of haemolysis is almost unavoidable in collecting blood, though this is often not evident until thick layers of plasma are examined spectroscopically for oxyhaemoglobin.

*Case I.* Specimens of blood from this case were sent by Dr. C. C. Ungley. The patient, a youth of 18 years, suffered from splenomegaly and severe haemolytic anaemia. The spleen was enlarged two fingers' breadth below the costal margin (Schüffner 1). A few days after admission, the red cells were 820,000 per c.mm., haemoglobin 20 per cent., colour index 1.2, reticulocytes 84 per cent., leucocytes 24,000 per c.mm., van den Bergh test, indirect positive (2.5 units), and mean corpuscular volume 128 c. $\mu$ . He had several transfusions, the last being on 26/4/39, by which time the red cells were 1,990,000 per c.mm., haemoglobin 44 per cent., colour index 1.1, and reticulocytes 61 per cent.

Special Laboratory Tests. Brownish-yellow plasma. Methaemalbumin demonstrable spectroscopically in a thickness of 3.0 cm.; Schumm's test was strongly positive. Van den Bergh test, direct negative; indirect positive (3.5 units).

Another case of haemolytic anaemia with splenomegaly might be included here though the condition developed on a chronic malarial splenomegaly acquired in childhood.

*Case II.* An Anglo-Indian man, aged 22 years, gave a history of recurrent malarial fever with splenomegaly and anaemia throughout childhood. A grave anaemia of haemolytic hypochromic type developed soon after his arrival in England (7/1/38).

Physical examination revealed pale mucous membranes, an enlarged heart, and a spleen extending to the umbilical level (Schüffner 2). Later, hepatomegaly, ascites, and generalized oedema due to temporary cardiac decompensation developed, but these features all disappeared with improvement in the anaemia.

Laboratory Investigations. Soon after admission to hospital (25/8/38) the red cells were 1,200,000 per c.mm., haemoglobin 15 per cent., colour index 0.6, and mean corpuscular volume 78.1 c. $\mu$ . The red cells showed hypochromia, anisocytosis, punctate basophilia, and Howell-Jolly bodies. Normoblasts and erythroblasts were noted. Van den Bergh test, indirect positive (4 units), direct negative. Methaemalbumin was present in a layer of serum 2 cm. thick. Schumm's test was strongly positive.

Progress. There was no haematopoietic response to iron medication and injections of large doses of reticulogen (a purified concentrated liver extract), but a marked response followed iron and crude liver extract therapy by mouth. Despite great improvement in the blood counts evidence of corpuscular destruction continued. Splenectomy was performed on 26/1/39 and the effects of operation on the anaemia, blood-pigments, and excretion of urobilinogen and porphyrin in the urine and faeces are summarized below. After operation a megalocytic anaemia developed, associated with a great increase in normoblasts, erythroblasts, and target cells, which constituted 47.5 per cent. of the erythrocytes in films taken on 21/11/39. There was also a great decrease in the corpuscular fragility to hypotonic saline, and though megalocytosis was marked (59 per cent.) and the corpuscular diameter had increased (8.924  $\mu$ ), the mean corpuscular thickness had decreased to 1.37  $\mu$  and the mean corpuscular volume was normal (85.7 c. $\mu$ ); these findings are all explicable in terms of the large number of target cells developing after the operation.

Methaemalbumin and Bilirubin. The effect of splenectomy on bilirubinaemia and methaemalbuminaemia may be studied in Fig. 1. Three days after the operation, methaemalbumin completely disappeared for a period of 10 days

or longer. There was also a reduction in the bilirubin content of the blood to 2 units. Later, both pigments reappeared in their former concentration. These findings suggested that splenectomy had decreased the production of lytic body, but that compensating hypertrophy of a pathological reticulo-endothelial system elsewhere in the body soon resulted in its reappearance with methaemalbumin formation.

**Urobilinogen and Porphyrin Excretion.** Dr. C. Rimington estimated the porphyrin excretion in the urine and faeces, and Dr. Janet Vaughan the urinary and faecal urobilinogen. The average faecal porphyrin excretion

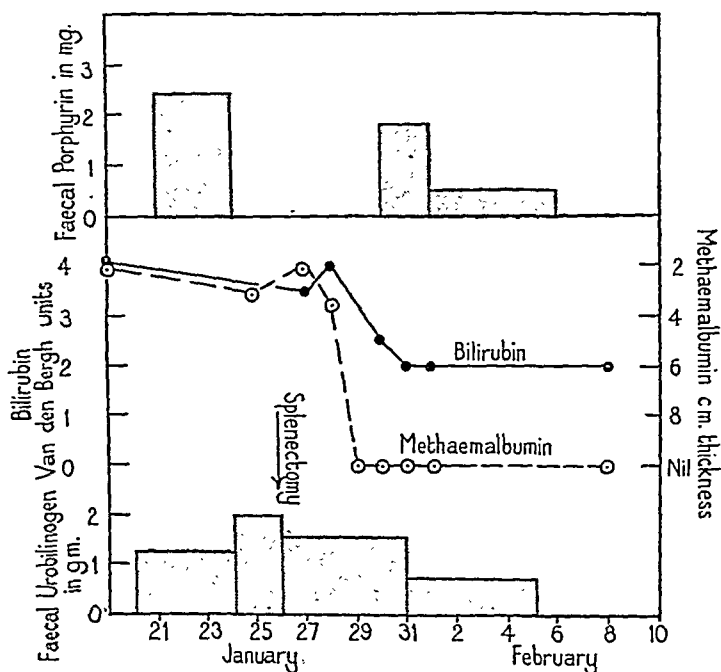


FIG. 1. Effect of splenectomy on the methaemalbumin and bilirubin values and the excretion of faecal porphyrin and faecal urobilin

over three days commencing on 21/1/39 was 2.45 mg. per diem, whereas the normal value is about 0.45 mg. Splenectomy was performed on 26/1/39. The average faecal porphyrin excretion over two days commencing on 30/1/39 was 1.83 mg. per diem, and for the next five days it had fallen to 0.49 mg. per diem which is almost normal (Fig. 1). The urinary porphyrin averaged 73.5  $\gamma$  for the two days preceding the operation. There was a rise to 133  $\gamma$  per diem for the four-day period following operation, but over the next six days the average was 45  $\gamma$  per diem which is within normal limits. Though the faecal urobilinogen excretion was not appreciably altered for the first five days after splenectomy, there was a significant reduction during the second five-day period. The value of 0.697 gm. per diem, however, was still almost three times the normal, and this was not unexpected, since after operation there had been only a decrease in the concentration of bilirubin in the plasma, and not a restoration to a normal level. With porphyrin excretion, on the other hand, normal values were reached in both faeces and urine and this was preceded by the disappearance of methaemalbumin from the blood. Such a result is in accord with the work of Rimington previously referred to, which suggested that methaemalbumin was a precursor of faecal porphyrin.



Progress. Throughout the year after operation, the red cells varied from 3,150,000 to 4,380,000 per c.mm., the haemoglobin from 46 to 60 per cent., and the colour index from 0.7 to 0.76 per cent. Treatment with iron and crude liver extract by mouth proved necessary from time to time. Were it not so expensive, a maintenance dose of both iron and liver would be preferable in cases of this type, since the intravascular blood destruction and resulting toxæmia must continually stimulate the red marrow elements to make good the loss, and for this purpose the necessary building materials for both haemoglobin and stroma must be required in large amounts.

*Haemolytic anaemia in malarial fever.* Malaria induces hypertrophy of the reticulo-endothelial system in general and of the spleen in particular; whatever degree of immunity is acquired in this disease is dependent on an enhanced ability of the reticulo-endothelial system to destroy free malarial parasites or parasitized corpuscles. The haemolytic anaemia accompanying acute malaria depends on a direct parasitic effect on the corpuscle, as in the febrile stage of malaria affecting man or in monkeys experimentally infected with *P. knowlesi*, where corpuscular destruction may be so great that haemoglobinaemia, and even haemoglobinuria may result. In patients with chronic malarial splenomegaly, on the other hand, malarial parasites may be difficult or impossible to demonstrate and malarial fever a rarity, yet haemolytic anaemia of normocytic, microcytic, or megalocytic type may be encountered. Reference will now be made to the findings only in acute febrile malaria, and not to chronic cases with 'ague-cake' spleen, as these form the subject of another communication.

An increase in the circulating bilirubin is well recognized in malarial fever at a time when parasites are demonstrable in peripheral blood smears. During the febrile period no significant haemoglobinaemia was found spectroscopically by Fairley and Bromfield (1933) in 32 cases. In a more recent series of five cases a positive Schumm's test was demonstrated in three malignant tertian infections and two benign tertian infections. Methaemalbumin, however, was never seen spectroscopically, even when a depth of plasma equalling 10 cm. was examined. In man, although some haemoglobin is probably liberated from the parasitized corpuscle when the merozoites escape in the circulation, the quantity is not sufficient to produce demonstrable haemoglobinaemia or methaemalbuminaemia, though it is sufficient to give rise to a positive Schumm's test. This corresponds with the experience of other observers who have reported so-called haematinaemia in malaria. Two factors contribute to the absence of demonstrable haemoglobinaemia. In the first place, by the time the schizogonous cycle is complete much of the haemoglobin contained in the parasitized corpuscle has been converted into malarial pigment, which Heilmeyer (1933) has shown to consist of haematin combined with some protein moiety. Secondly, some of the parasitized corpuscles are engulfed by the hypertrophied reticulo-endothelial system before rupture has occurred; in such circumstances the katabolism of haemoglobin is intracellular and would follow the verdohaemochromogen-biliverdin route to bilirubin, the iron moiety presumably being converted into

haemosiderin and subsequently being deposited as such in the parenchymatous cells of the viscera, especially the liver, spleen, and kidney.

*Pernicious anaemia.* The fact that Schumm's test is positive in pernicious anaemia is well recognized. Schumm (1932) found it frequently in 100 cases and Bingold (1930) concluded that if repeated examinations were made haematin could always be detected in the blood-serum by this means. The test became negative after complete remission induced by liver extract therapy. Three typical untreated cases of pernicious anaemia were examined in the present series. The red-cell counts varied between 1,370,000 and 1,830,000 per c.mm., the haemoglobin from 46 to 52 per cent., and the colour index from 1.2 to 1.5. Van den Bergh test, indirect positive (1.5 to 1.75 units). In no instance was methaemalbumin demonstrable spectroscopically, but all gave a positive Schumm's test in a depth of plasma varying from 0.5 to 1.5 cm. In a fourth patient who had received liver extract therapy for 12 days, the plasma contained 2.25 units of bilirubin (indirect reaction), gave a negative Schumm's test, and showed no methaemalbumin on spectroscopical examination. Prior to treatment the red cells were 1,370,000 per c.mm., the haemoglobin 36 per cent., and the colour index 1.3 per cent.

*Group III. Haemolytic anaemia with hyperbilirubinaemia.* In cases of splenomegaly associated with latent chronic malaria examined between the febrile paroxysms, hyperbilirubinaemia may be found in the plasma showing a negative Schumm's test and no demonstrable methaemalbumin. In other cases hyperbilirubinaemia is absent. In acholuric family jaundice Bingold (1930, 1932) has reported negative Schumm's tests in the plasma, associated with the hyperbilirubinaemia characteristic of this form of haemolytic icterus. In the present investigation three cases of acholuric family jaundice with typical family histories and splenomegaly were investigated. All showed an increased corpuscular fragility to hypotonic saline. The red-cell counts varied between 1,600,000 and 3,600,000 per c.mm., the haemoglobin from 34 to 70 per cent., and the colour index from 0.9 to 1.0. Reticulocyte counts in two cases were 14 and 15 per cent., the third was not available. The bilirubin content of the plasma was 2.5, 4.5, and 8.5 units. In no instance was methaemalbumin demonstrable spectroscopically. In the first two cases the plasma yielded a negative Schumm's test; in the third it was weakly positive, the  $\alpha$  band of the haemochromogen being visible only through a depth of plasma equalling 2.5 cm. The fact that in pernicious anaemia Schumm's test is so frequently and strongly positive, and that in acholuric family jaundice the test is negative or only weakly positive, is of considerable interest. It suggests that in pernicious anaemia destruction of the red cells is occurring in the circulating blood, whereas in acholuric jaundice, despite the increased fragility of the corpuscles to hypotonic saline solution, the blood destruction is predominantly within the reticulo-endothelium of the spleen.

*Summary and Conclusions*

(1) The spectroscopic appearances and chemical reactions of methaemalbumin in human plasma and when concentrated in the albumin fraction of blackwater fever serum are described.

(2) Simple chemical methods used in conjunction with a direct vision spectroscope are described which serve to differentiate methaemalbumin from methaemoglobin and sulphaemoglobin.

(3) Methaemoglobin has been universally described in the plasma of diseases associated with intravascular haemolysis and haemoglobinuria. This error has arisen through failure to differentiate spectroscopically between methaemoglobin and methaemalbumin.

(4) As methaemoglobin and sulphaemoglobin are essentially confined within the corpuscle it is suggested that the term methaemoglobincythaemia should replace that of methaemoglobinaemia, and sulphaemoglobincythaemia that of sulphaemoglobinaemia.

(5) Since it has been shown that haematin (ferrie) in plasma immediately unites with the serum albumin to form methaemalbumin, Schumm's test in plasma is to be regarded as essentially a test indicating the presence of methaemalbumin, and not free haematin as has previously been supposed.

(6) It follows that the condition previously described in medical literature as haematinaemia on the basis of this test should henceforth be called methaemalbuminaemia.

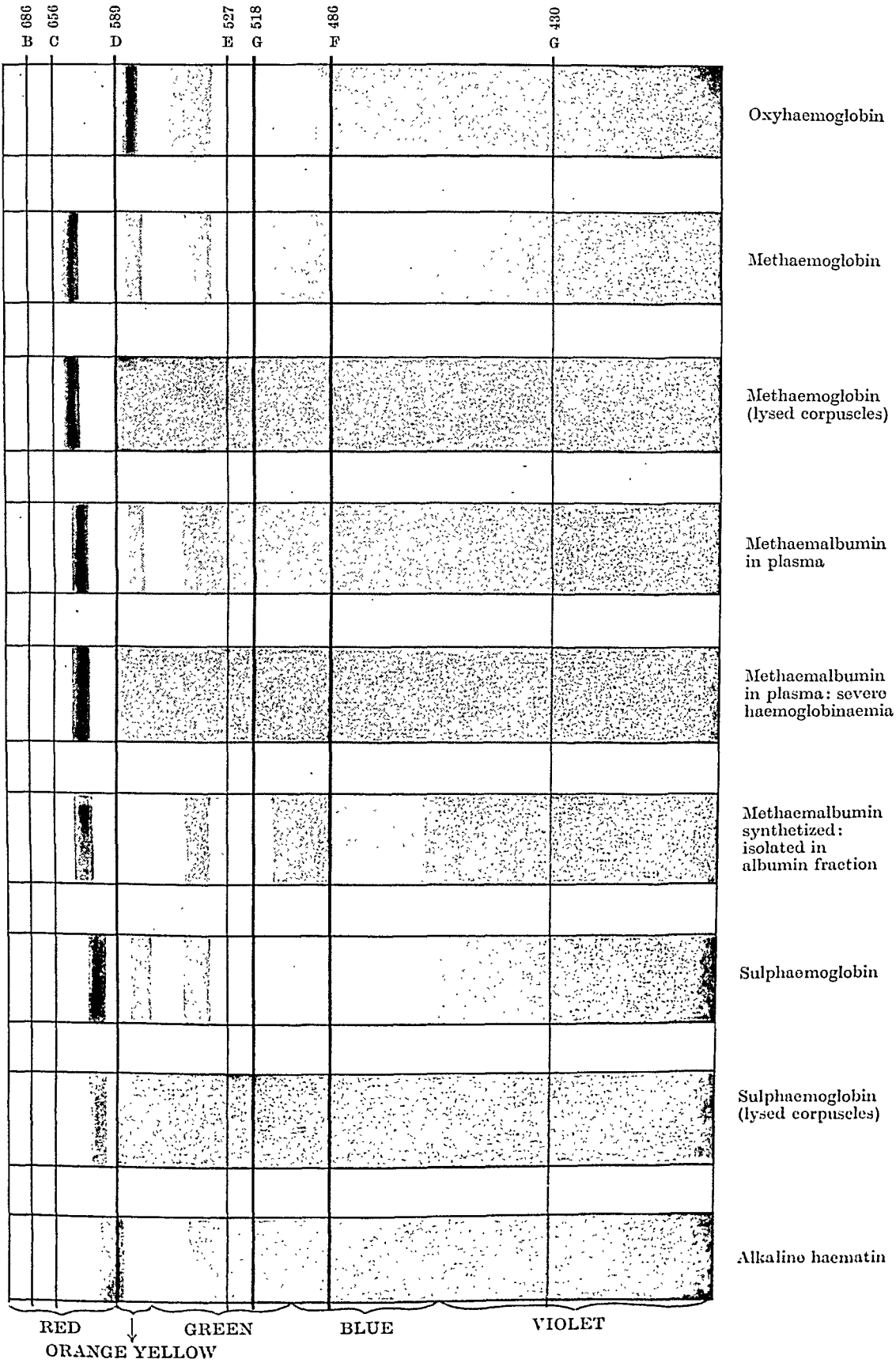
(7) From a biochemical viewpoint the haemolytic anaemias fall naturally into three groups accordingly as they show: (a) hyperbilirubinaemia alone, (b) hyperbilirubinaemia and methaemalbuminaemia, and (c) hyperbilirubinaemia, methaemalbuminaemia, and haemoglobinaemia.

(8) Regarding the site of haemolysis the available evidence suggests that hyperbilirubinaemia associated with a negative Schumm's test is indicative of intracellular blood destruction, whereas methaemalbuminaemia implies lysis of corpuscles in the circulating blood.

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# METHAEMALBUMIN<sup>1</sup>

## PART II. ITS SYNTHESIS, CHEMICAL BEHAVIOUR, AND EXPERIMENTAL PRODUCTION IN MAN AND MONKEYS

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### Introduction

IN the preceding paper (Fairley, 1941) the methods of identification of methaemalbumin (pseudo-methaemoglobin) in plasma and its clinical significance were considered. In the present paper a detailed account of the original experiments dealing with the synthesis of methaemalbumin are recorded, along with the additional experimental data concerning the chemical behaviour of this pigment *in vitro* and its production *in vivo* by intravenous injections of haematin (ferric) in man and monkeys.

A brief review of the relevant literature and the various stages in the investigation may be summarized as follows. Fairley and Bromfield (1934) described in blackwater fever a previously unidentified pigment with an  $\alpha$  band at  $623\ \mu\mu$ , which formerly had been regarded by all observers as methaemoglobin. Apart from the spectroscopic picture and other differences, the  $\alpha$  band of the new pigment was not dispersed by Stokes's reagent or 10 per cent. ammonium sulphide, but was dispersed with strong ammonium sulphide with the formation of a haemochromogen having an  $\alpha$  band at  $558\ \mu\mu$ . Owing to certain resemblances to methaemoglobin, this new pigment, which in the meantime had been demonstrated in several different types of haemoglobinuria, was named pseudo-methaemoglobin by Fairley (1937). Foy and Kondi (1938) in a spectrographic analysis of pigments in serum from blackwater-fever patients, confirmed these findings and found pseudo-methaemoglobin in the plasma in the majority of cases of blackwater fever examined in Greece. Spectrograms showed that the absorption maximum of the band in the red varied from  $622\ \mu\mu$  to  $624\ \mu\mu$ .

It had been well recognized that on incubation solutions of haemoglobin yielded methaemoglobin and later haematin, but Fairley and Bromfield (1937) observed that when oxyhaemoglobin was incubated at  $40^\circ\text{C}$ . with human plasma, pseudo-methaemoglobin and not neutral methaemoglobin<sup>2</sup>

<sup>1</sup> Received July 13, 1940.

<sup>2</sup> More recent experiments have shown that after incubation neutral methaemoglobin is also produced for the first few days, but owing to a change in pH to the alkaline side of 8.0 it tends to be converted into alkaline methaemoglobin, its presence thus being masked unless the pH be readjusted with acid.

was found spectroscopically after 48 hours. Similarly, when alkaline haematin (ferric) was added to human plasma or serum, pseudo-methaemoglobin immediately formed. This suggested that the haematin was coupling with some protein or other nitrogenous constituent of the plasma to form the new pigment.

Heilmeyer (1933), who had investigated by spectrophotometry the absorption curves of alkaline haematin added to serum, also came to the conclusion that alkaline haematin must be combining with some protein constituent which required strong alkali to split it, and that this compound was responsible for the distinctive spectrum which he showed differed definitely from that of alkaline haematin. The conclusions reached from the author's clinical and experimental observations were arrived at independently and without knowledge of Heilmeyer's *in vitro* studies, which appear to have attracted little or no attention by writers on haematinaemia either at the time or since. When it was later discovered that pseudo-methaemoglobin could be synthesized by the addition of alkaline haematin (ferric) to the albumin fraction of human serum and to crystalbumin, Fairley (1938) renamed the pigment methaemalbumin. This had become additionally desirable since, in the interim, Barkan and Schales (1937) had some months after Fairley and Bromfield's publication, applied the name 'pseudomethaemoglobin' (unhyphenated) to an entirely different pigment.

#### *Experiments with Alkaline Haematin and Plasma*

After the incubation experiments with plasma and haemoglobin cited above, an alkaline solution of commercial haematin (British Drug Houses) was similarly incubated in the presence of human plasma. In less than 30 min. methaemalbumin (pseudo-methaemoglobin) was formed. The haematin used in this particular experiment was not a purified preparation, and in all subsequent experiments only specially purified haemin was used as a source of alkaline haematin (ferric). In the original experiments with the purified product (16/7/37), haemin (1 mg. per 3 c.c.) was dissolved in distilled water made alkaline with a few drops of 10 per cent. sodium hydroxide. Sufficient of this was added to 5 c.c. of serum or plasma to give a well-defined  $\alpha$  band in the red portion of the spectrum. This band proved to be collinear with that of methaemalbumin (pseudo-methaemoglobin) at  $623\mu$  on the Hartridge reversion spectroscope, and gave other chemical reactions regarded as being characteristic of this pigment.

These results were confirmed with human serum and plasma, and a number of different mammalian sera were also investigated. Where the serum under investigation had been kept at  $4^{\circ}\text{C}$ . it was found advisable to warm it to  $37^{\circ}\text{C}$ ., as otherwise the formation of methaemalbumin (pseudo-methaemoglobin) might be delayed. In the initial experiments the distilled water was made just sufficiently alkaline with a few drops of 10 per cent. sodium hydroxide to dissolve the haematin completely. To avoid excess of



the latter pigment only a small amount of alkaline haematin (ferric) was added to each sample of serum (at 37°C.) in the first instance, and the mixture was then examined spectroscopically. If no well defined spectrum had developed, alkaline haematin was subsequently added, a few drops at a time, until this had been achieved. Then the resulting pigment was examined spectroscopically on the Hartridge reversion spectroscope to see if the  $\alpha$  band was collinear with that of methaemalbumin (pseudo-methaemoglobin) or whether the spectrum corresponded to that of alkaline haematin. The results obtained in one such experiment are incorporated in Table I.

It will be seen that only human and simian serum in the presence of alkaline haematin proved capable of producing methaemalbumin (pseudo-methaemoglobin), and that in the case of all other animal sera tested a haematin-like

TABLE I

*The Effect of Adding Alkaline Haematin (Ferric) to the Serum of Different Species of Animals and Man*

Source of serum	Pigment found	
	Methaemalbumin (Pseudo-methaemoglobin) ( $\alpha$ band at 623 $\mu\mu$ )	Haematin-like spectrum
Man	+	0
<i>Cercopithecus aethiops</i>	+	0
<i>Macacus iris</i>	+	0
<i>Macacus rhesus</i>	+	0
<i>Macacus sinicus</i>	+	0
Pig	0	+
Dog	0	+
Horse	0	+
Sheep	0	+
Rabbit	0	+
Guinea-pig	0	+
Cat	0	+

spectrum persisted. More recently, to ensure a constant concentration of pigment for experimental work, weighed quantities of purified haemin have been dissolved in N/100 sodium hydroxide (1 mg. per c.c.). One volume of this alkaline haematin (ferric) solution has been added to five volumes of serum or plasma (pH = 7.8 to 8.0) with the production of a clear brown methaemalbumin solution having a pH of from 8.0 to 8.3. Similar findings were obtained when haemoglobin was incubated in the presence of different mammalian sera at 40°C., for after three days methaemalbumin (pseudo-methaemoglobin) with an  $\alpha$  band at 623  $\mu\mu$  was demonstrable only with human and simian, but not with other mammalian sera. The conclusion reached was that alkaline haematin (ferric), or haematin formed from incubated haemoglobin, was combining with some protein or other nitrogenous constituent contained in human or simian plasma or serum to form methaemalbumin (pseudo-methaemoglobin), but that this coupling did not occur in other mammalian sera examined.

*Haematin and the Albumin Fraction of Sera*

Initial investigations, undertaken to determine the nature of the unknown plasma constituent, yielded negative results when solutions of alkaline haematin (ferric) were added to crystalline horse albumin, the pseudo-globulin and euglobulin fractions of human serum, and numerous nitrogenous constituents of human serum. Later, serum protein fractions from man and monkey were prepared by Dr. C. Rimington, and to these were added an alkaline solution of haematin (ferric) prepared from pure haemin, until a well-developed spectrum was evident. Equine plasma and a solution of crystalline horse albumin were used as controls. The results are summarized in Table II.

In the presence of alkaline haematin, the whole serum and the albumin fractions derived from man and *Cercopithecus aethiops* all formed methaemalbumin (pseudo-methaemoglobin) whereas equine plasma, crystalline horse

TABLE II

*The Addition of Alkaline Haematin to the Protein Fractions of Human, Simian, and Equine Sera*

Species	Whole serum	Fractionated serum		
		Albumin	Euglobulin	Pseudo-globulin
Man	+	+	0	0
<i>Cercopithecus aethiops</i>	+	+	0	0
Horse	0	0	-	-

+ = methaemalbumin (pseudo-methaemoglobin).

albumin, and the human euglobulin and pseudo-globulin fractions failed to do so. These results suggested that the constituent coupling with haematin to form methaemalbumin (pseudo-methaemoglobin) was contained in the albumin fraction of plasma derived from man and *Cercopithecus aethiops*.

The results of these original experiments have been repeatedly confirmed during the past two years with albumin and globulin fractions separated from human and simian sera; pigment with the typical spectrum and chemical reactions of methaemalbumin (pseudo-methaemoglobin) has invariably resulted on addition of alkaline haematin (ferric) to the serum albumin fraction. The fractions have always been dialysed in running water for several days and finally in distilled water until all traces of ammonium sulphate have disappeared. Sometimes the fraction has been examined in distilled water and at other times it has been finally dialysed against a phosphate buffer solution at a pH of 8.0, but in either case the pigment with an  $\alpha$  band at  $623\mu$  giving the chemical reactions of methaemalbumin (pseudo-methaemoglobin) has been formed only in the haematin-albumin fraction and never in the euglobulin or pseudo-globulin fractions.

When rabbit serum was fractionated by a similar technique and a solution of alkaline haematin (ferric) added to the albumin, euglobulin, and pseudo-

globulin fractions, a haematin-like spectrum was formed in all three fractions and no spectroscopic evidence of the formation of methaemalbumin (pseudo-methaemoglobin) was forthcoming.

The conclusion reached from these and other investigations was that haematin was combining with some constituent (presumably serum-albumin) contained in the human and simian albumin fraction to form methaemalbumin (pseudo-methaemoglobin), but that this coupling did not occur in the albumin fraction of rabbit serum and other mammalian sera of similar type. Further investigations, especially those regarding the behaviour of rabbit serum-haematin mixtures in the ultracentrifuge have, however, thrown doubt on the validity of this conclusion and indicate that in the presence of rabbit serum, despite the spectroscopic similarity to alkaline haematin, the haematin does combine with serum-albumin and is not in a free state. Reference to these experiments will be made later.

*The Synthesis of Methaemalbumin (Pseudo-Methaemoglobin) from  
Haematin (Ferric) and Crystalalbumin*

Hewitt (1936, 1937, 1938) has shown that the albumin fraction of human serum contains several different proteins, notably crystalalbumin, globoglycoid, and seroglycoid. It appeared advisable to determine which of these constituents was combining with alkaline haematin (ferric) to form the new pigment, and the results of three experiments are summarized in Table III. Dr. L. F. Hewitt supplied solutions of the three proteins prepared from the

TABLE III

*The Addition of Alkaline Haematin to the Various Proteins Found in the  
Albumin Fraction of Human Serum*

Source of proteins	Proteins in the albumin fraction		
	Crystalalbumin	Globoglycoid	Seroglycoid
Human serum	+	0	0
Horse serum	0	0	0
Human serum	+	?	-

albumin fraction of human serum used in the first experiment and they were tested in a concentration approximating to that found in normal serum; the pH of the crystalalbumin and globoglycoid solutions was approximately 7.0 and that of the seroglycoid 6.0. When alkaline haematin prepared by dissolving pure haemin in normal sodium carbonate solution was added to these various protein solutions in a concentration sufficient to yield a well defined spectrum, it was found that methaemalbumin had formed only with crystalalbumin, and not in the presence of globoglycoid or seroglycoid where the spectrum of alkaline haematin remained apparently unmodified.

In the second experiment, specimens of equine crystalalbumin (pH = 5.4), equine globoglycoid, and equine seroglycoid (pH = 5.5), which had been

prepared by Dr. C. Rimington, were also investigated along similar lines. The alkaline haematin ( $\text{pH} = 9.5$ ) was prepared by dissolving 1 mg. of purified haemin in 1 c.c. of  $\text{N}/100$  sodium hydroxide, and this was subsequently added to solutions of these proteins isolated from the albumin fraction of horse serum in a quantity sufficient to give a well-defined spectrum. Methaemalbumin was never produced. The spectroscopic appearance and the chemical behaviour resembled that of haematin. Further, on varying the conditions of  $\text{pH}$  the alterations in the position of the  $\alpha$  band corresponded to those observed in a protein-haematin mixture, and not to that found with the more stable methaemalbumin compound. On the other hand, in a specimen of human crystalalbumin tested a few days later, as well as with the original albumin fraction of human serum from which it had been prepared, the addition of alkaline haematin immediately resulted in the production of methaemalbumin, giving the typical spectroscopic appearance and chemical reactions described in the following section. When alkaline haematin was added to the human globoglycoid solution, also prepared by Dr. C. Rimington, a spectrum was produced closely resembling that of methaemalbumin ( $\text{pH} = 6.3$ ), but on repeating this two days later both the spectroscopic appearances and chemical behaviour resembled those of haematin, not methaemalbumin. Evidence, however, was obtained that the protein was undergoing denaturation so it will be necessary to repeat this observation at a later date with freshly prepared human globoglycoid before coming to any conclusion on this point. This is especially desirable since Rimington (personal communication) finds no significant chemical or immunological differences between crystalalbumin and globoglycoid, and attributes any different properties they possess to an altered physical state of the albumin in the two compounds.

*Spectrum of synthesized methaemalbumin.* The spectrum of the pigment obtained by adding haematin (ferric) to the albumin fraction of human serum and to crystalalbumin was studied on the reversion spectroscope in solutions entirely free from haemoglobin, the presence of which in specimens of plasma or serum obtained from man so often complicates the spectroscopic picture. Three bands were generally evident, a well-defined  $\alpha$  band at  $623\ \mu\mu$  to  $624\ \mu\mu$ , a  $\beta$  band at  $539\ \mu\mu$  to  $541\ \mu\mu$ , and a  $\gamma$  band  $500\ \mu\mu$  to  $501\ \mu\mu$  superimposed on a general absorption. In some samples it was not possible to define clearly the  $\beta$  and  $\gamma$  bands as there was a general absorption commencing about the position of the  $\beta$  band and extending towards the short wave length of the spectrum. The positions of these three bands as determined by the reversion spectroscope correspond to those observed in the albumin fraction of blackwater-fever serum, but differ somewhat from those of Heilmeyer (1933) who investigated by spectrophotometry the absorption curves of alkaline haematin (ferric) added to serum. He found an absorption curve in the red with a maximum situated at  $620\ \mu\mu$  and an absorption in the green with a double-headed maximum at approximately  $535\ \mu\mu$  and  $495\ \mu\mu$ ; from  $470\ \mu\mu$  there was a steep final rise in the blue. The differences

observed in the positions of these bands evidently depend on the different methods used in their determination; in the routine examination of methaemalbumin in human plasma, the  $\alpha$  band is constantly found between  $623\ \mu\mu$  and  $624\ \mu\mu$  by the reversion spectroscope and between  $622\ \mu\mu$  and  $624\ \mu\mu$  in spectrograms, as the studies of Foy and Kondi (1938) have shown.

*Chemical reactions of synthesized methaemalbumin.* The reactions used in the routine differentiation of methaemalbumin from methaemoglobin and sulphaemoglobin in blood were also investigated on the pigment synthesized from both the albumin fraction and crystalalbumin. It was found that both Stokes's reagent (2 drops per c.c.) and 10 per cent. ammonium sulphide (1 drop per c.c.) failed to disperse the  $\alpha$  band of synthesized methaemalbumin just as they had in the case of this pigment in blackwater-fever plasma. On the addition of strong ammonium sulphide, the band in the red was rapidly dispersed and a haemochromogen formed with an  $\alpha$  band situated at  $558\ \mu\mu$  (Schumm's test).

*Reduction with sodium hydrosulphite ( $\text{Na}_2\text{S}_2\text{O}_4$ ) to form haemalbumin.* On reduction with sodium hydrosulphite the colour changed from brown to red; in a concentrated solution of methaemalbumin prepared by the addition of alkaline haematin to human plasma or the albumin fraction of human serum a broad-banded spectrum somewhat resembling reduced haematin became apparent, but on dilution this resolved into a two-banded spectrum with a prominent  $\alpha$  band at  $573$  to  $574\ \mu\mu$  and a  $\beta$  band at  $529$  to  $532\ \mu\mu$ . With methaemalbumin synthesized from human crystalalbumin and alkaline haematin, the  $\alpha$  band varied from  $570$  to  $571\ \mu\mu$  and the  $\beta$  band from  $530$  to  $531\ \mu\mu$ . These spectroscopic findings corresponded closely with those observed when the pigment concentrated in the albumin fraction of blackwater-fever serum was reduced with sodium hydrosulphite, a prominent  $\alpha$  band being situated at  $571$  to  $572\ \mu\mu$  and a weaker  $\beta$  band at  $531$  to  $532\ \mu\mu$ . In all instances, the spectrum was attributed to the formation of a new compound, haemalbumin, containing ferrous iron. It differed from the purplish brown colour and broad diffuse spectrum with a suggestion of two bands presented by reduced haematin (ferrous) when colloidal alkaline haematin (ferric), prepared from purified haemin, is reduced with sodium hydrosulphite. When excess of sodium hydrosulphite is added to synthesized methaemalbumin, the  $\beta$  band fades after a few minutes and a single diffuse band persists, but in these circumstances there is evidence of actual disintegration of the original compound and precipitation of protein, the reduced haematin probably being split off from its albumin component. When synthesized methaemalbumin solution was reduced with sodium hydrosulphite, subsequently depleted of oxygen by a Geryk vacuum pump, and kept at  $16^\circ$  to  $18^\circ\text{C}$ . overnight, spectroscopic examination revealed a three-banded spectrum, a broad diffuse  $\alpha$  band at  $572\ \mu\mu$ , a well-defined  $\beta$  band at  $551\ \mu\mu$ , and a hazy narrow  $\gamma$  band at  $524\ \mu\mu$ .

Colloidal haematin (ferric) with a pH of 8.7 was used as a control. For its preparation, purified haemin was dissolved in N/100 sodium hydroxide and

added to 0.4 per cent. gum acacia in a phosphate buffer solution at pH 8.0. This was treated with excess of sodium hydrosulphite with the production of reduced haematin, and the tube containing the pigment was exhausted of air, sealed, and allowed to stand overnight. Next morning the pH of the solution was 6.0 and spectroscopic examination revealed a diffuse absorption band at  $637\mu\mu$  due to the formation of acid haematin. It appears from these observations that on reduction of methaemalbumin with sodium hydrosulphite a new compound, haemalbumin, is produced characterized by a two-banded spectrum, and that a diffuse single-banded spectrum resembling reduced haematin is evident only after this compound has undergone disintegration. The identity of the compound or compounds responsible for the three-banded spectrum formed after treatment with sodium hydrosulphite and depletion of oxygen was not determined.

*Sodium hydrosulphite and 10 per cent. sodium hydroxide.* On treating synthesized methaemalbumin with sodium hydrosulphite and 10 per cent. sodium hydroxide, a haemochromogen is formed which is indistinguishable from globin-protohaemochromogen. When the pigment has been produced by the addition of alkaline haematin (ferric) to the albumin fraction of human serum it is probable that denatured seroglycoid, globoglycoid, and crystalalbumin all combine with reduced haematin (ferrous) to form haemochromogens. When the pigment is produced by the addition of alkaline haematin to a solution of crystalalbumin, which contains about 95 per cent. of pure serum-albumin, the haemochromogen formed must consist of almost pure albumin-haemochromogen.

*Sodium hydrosulphite and carbon monoxide (carboxyhaemalbumin).* Similarly, when synthesized methaemalbumin, prepared from either the albumin fraction or human crystalalbumin, was reduced with sodium hydrosulphite and carbon monoxide subsequently bubbled through the solution, a cherry red colour was produced. Spectroscopic examination showed a prominent two-banded spectrum very similar to that of carboxyhaemoglobin. Where the methaemalbumin was made from crystalalbumin this was attributed to the formation of a corresponding compound, carboxyhaemalbumin, which resembled carboxyhaemoglobin more closely than carboxy-reduced-haematin.

*Comment.* The identical nature of the spectrum and the similarity of the chemical behaviour of the pigment synthesized by the addition of alkaline haematin (ferric) to the human serum-albumin fraction or to human crystalalbumin leave no doubt as to the identity of this haematin-albumin compound, methaemalbumin, with the similar pigment concentrated in the albumin fraction of blackwater-fever serum. Though the spectroscopic appearances and chemical reactions of methaemalbumin are not in all respects analogous to those of methaemoglobin, the available data, including that cited later, indicate that in methaemalbumin the prosthetic group is oxidized haematin containing trivalent or ferric iron, while the protein component is native serum-albumin instead of native globin. According to Anson and Mirsky (1925) the capacity of chromoproteins like haemoglobin

and heliocorubin to combine loosely with oxygen is a property possessed only when they are polymerized. The failure of haemalbumin to combine loosely with oxygen may be dependent on the absence of polymerization, or it may be due to the different protein component, that is, albumin instead of globin.

*Effect of Absolute Alcohol, Acid, and Alkalis on Methaemalbumin*

*Absolute alcohol.* If to synthesized methaemalbumin alcohol be added in increasing concentration there is first a precipitation of protein which does not apparently include methaemalbumin. On increasing the concentration to 40 per cent. practically all the pigment comes down in the brown precipitate obtained on centrifugalization, while the supernatant fluid, though still very slightly brown, shows no spectroscopic evidence of methaemalbumin. A concentration of 60 per cent. alcohol leads to further precipitation of protein and to complete clearing of the supernatant fluid, which gives a negative Schumm's test and shows no spectroscopic evidence of pigment. Methaemalbumin along with the other serum proteins, is irreversibly precipitated; the pigment is not recoverable from the centrifugalized deposit.

*Alkali.* When to the synthesized methaemalbumin solution ( $\text{pH} = 8.3$ ), prepared as usual from alkaline haematin (ferric) and plasma, excess of 10 per cent. sodium hydroxide is added drop by drop, the  $\alpha$  band in the red is dispersed. On the addition of sufficient hydrochloric acid to neutralize this excess of soda, spectroscopic examination again shows the spectrum of methaemalbumin with an  $\alpha$  band at  $623 \mu\mu$ . On reduction with sodium hydrosulphite the two-banded spectrum of haemalbumin with an  $\alpha$  band at  $568 \mu\mu$  and a  $\beta$  band at  $528 \mu\mu$  appears, and on adding 10 per cent. sodium hydroxide a typical haemochromogen is formed with an  $\alpha$  band at  $558 \mu\mu$ .

*Acid.* When normal hydrochloric acid is added drop by drop to a similar solution of synthesized methaemalbumin, the  $\alpha$  band is dispersed and acid haematin formed; if excess be added the  $\alpha$  band of acid haematin disappears. On neutralization with a corresponding quantity of normal sodium hydroxide spectroscopic examination shows that methaemalbumin has reformed.

*Comment.* Though the addition of excess of alkali or acid leads to the dispersion of the spectrum of methaemalbumin, the reaction is reversible, since on neutralization spectroscopic examination shows that methaemalbumin has reappeared.

*The Effect of Changes of pH on the Spectrum of Various Haematin Mixtures*

Heilmeyer (1933) drew attention to the prevailing opinion that haematin or haemin, dissolved in blood serum, possessed the same spectrum as that of alkaline haematin, whereas in fact there were considerable differences. The question arose as to whether variation in hydrogen-ion concentration might explain the difference. To test this possibility the absorption curves of haemin, dissolved in borate buffer solutions of  $\text{pH } 8.1, 9.0, 10.0$ , and  $11.35$  were worked out by Heilmeyer. The results showed that these absorption

curves were clearly dependent on hydrogen-ion concentration, whereas the curve of haemin dissolved in serum fell completely out of this group, despite the fact that its pH lay between 9.0 and 10.0. Furthermore, when the alkaline serum-haematin solution was neutralized by the introduction of carbon dioxide, the characteristic absorption curve with its maximum at  $620\mu\mu$  remained unaltered. Only when an excess of strongly concentrated alkali was added was the serum spectrum carried over to that of alkaline haematin at  $610\mu\mu$ . Heilmeyer concluded from these *in vitro* experiments that, when added to serum, haematin must combine with some protein constituent which requires strong alkali to split it, and that this compound was responsible for the distinctive spectrum which differs so definitely from that of alkaline haematin. In order to obtain more data on this subject it was decided to investigate the effect of changes of pH on the wave length of the band in various haematin mixtures including colloidal haematin, haematin plus recrystallized egg albumin, haematin plus human serum, haematin plus the globulin fraction, and haematin plus albumin fraction of human serum.

*Methods.* For this purpose purified haemin was dissolved in N/100 sodium hydroxide in a concentration of 0.5 to 1 mg. per c.c., one volume of alkaline haematin being added to three volumes of the particular solution under investigation. The colloidal haematin was prepared by adding one volume of alkaline haematin solution to three volumes of 0.4 per cent. gum acacia in phosphate buffer solution at pH 7.8. The egg albumin, which had been recrystallized several times, was kindly supplied by Dr. R. A. Kekwick. It was dialysed against running water for several days to remove ammonium sulphate, then against distilled water, and finally against phosphate buffer solution at pH 8.0. It was then diluted with buffer solution to give approximately a 4 per cent. solution, and one volume of alkaline haematin (1 mg. per c.c.) was added to three volumes of egg-albumin solution. The albumin and globulin fractions of human serum were fractionated by the ammonium sulphate method, and after being dialysed against running and distilled water were finally dialysed against phosphate buffer solution (pH = 7.8). These were then diluted with this solution to the original volume of serum used in their preparation. One volume of alkaline haematin (1 mg. per c.c.) was added to three volumes of the globulin fraction, and one volume of alkaline haematin (0.5 mg. per c.c.) to three volumes of the albumin fraction and also to three volumes of human serum which had stood in contact with clot over-night (pH = 8.17). The reason for adding only 0.5 mg. of haematin per c.c. of the serum and to the human albumin fraction was because the resulting spectrum of methaemalbumin was too dense for reading the position of the  $\alpha$  band on the reversion spectroscope if the larger amount of haematin were added. With the other mixtures the smaller amount of haematin failed to give a satisfactory spectrum and the amount had to be doubled. To a series of test-tubes containing a unit volume of each of these haematin mixtures were added N/10 to N/100 hydrochloric acid and N/10 sodium hydroxide in varying quantities so as to give a wide range of pH varying from 2 to 11. After the position of the  $\alpha$  band in the various haematin mixtures had been determined on the Hartridge reversion spectroscope, the pH of each of the samples as well as of the original haematin mixture was determined by Dr. J. C. Broom using a glass-electrode. The apparatus, which makes use of a special spiral electrode, was designed



by Dr. C. G. Pope of the Wellcome Physiological Research Laboratories and is manufactured by Unicam Instruments Ltd., Cambridge.

*Results.* The results are shown in Fig. 1. With the human serum-haematin mixture it will be seen that the  $\alpha$  band at  $623\mu\mu$  remained unaltered over a wide range of pH varying from 4.7 to 11.0. At pH 4.2 the  $\alpha$  band showed displacement towards the long-wave end of the spectrum and was situated at  $625\mu\mu$ . The position of the band then changed rapidly with slight alterations in pH, being  $641\mu\mu$  at pH 3.5. Turbidity was noted in the solutions

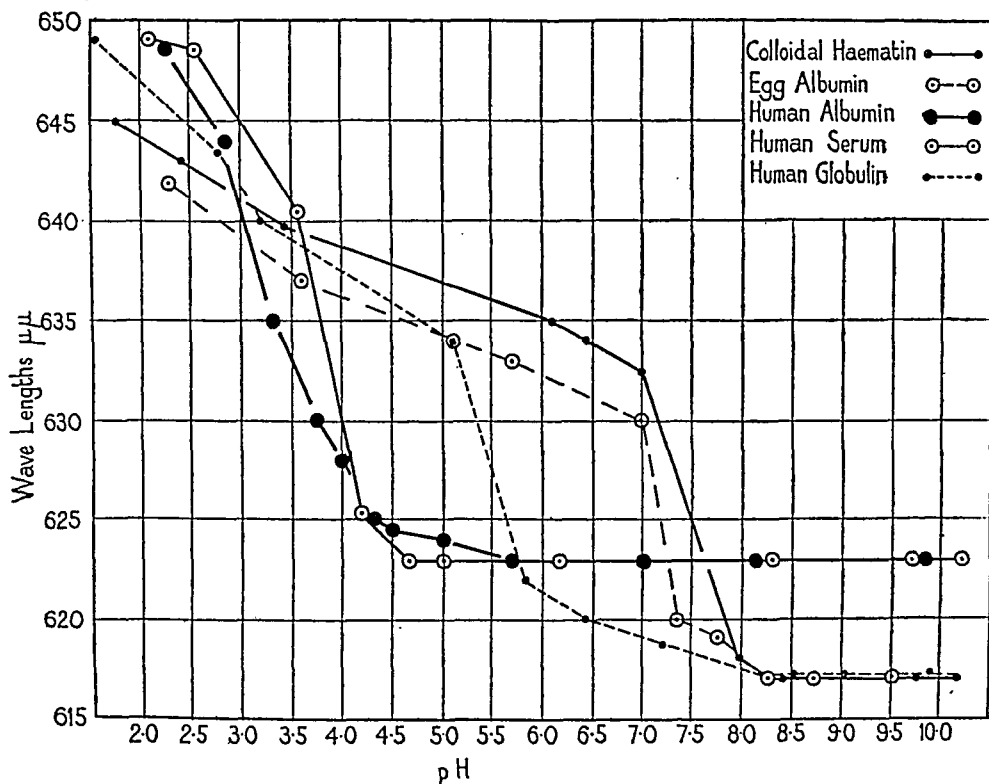


FIG. 1. The effect of varying the pH of colloidal haematin and certain haematin-protein mixtures on the position of the  $\alpha$  band

between pH 4.0 and 6.0, and with increasing acidity below pH 4.0 the band in the red portion of the spectrum became diffuse and difficult to read. A very similar graph was found with the human albumin fraction-haematin mixture. Thus the  $\alpha$  band remained unaltered at  $623\mu\mu$  over a range of pH of from 5.7 to 11.0. At pH 5.0 a slight shift to  $624\mu\mu$  was noted and at pH 4.3 it had changed to  $625\mu\mu$ . Thereafter only a slight decrease in pH resulted in marked displacement of the  $\alpha$  band towards the long-wave end of the spectrum as shown in Fig. 1. At pH 3.3 it was  $635\mu\mu$  and at pH 2.85 it was  $644\mu\mu$ . Again, with increasing acidity, the  $\alpha$  band in the red became broader and more diffuse, causing difficulty in making satisfactory readings.

With the colloidal haematin, the recrystallized egg albumin-haematin mixture, and the globulin fraction-haematin mixture an entirely different

type of graph resulted. Between a pH of 8.3 to pH 11.0 they all showed the spectrum of alkaline haematin with a broad  $\alpha$  band situated about  $617\mu\mu$ . About a pH of 8.3 the addition of acid in each case led to an alteration in the position of the  $\alpha$  band which was displaced towards the long-wave end of the spectrum. With colloidal haematin the displacement was considerable. At pH 7.9 the  $\alpha$  band was at  $618\mu\mu$  and at 7.0 it was at  $632\mu\mu$ , thereafter changing more slowly (Fig. 1). A somewhat similar curve was obtained with the buffered egg albumin-haematin mixture; at pH 7.7 the  $\alpha$  band was situated at  $619\mu\mu$ , at pH 7.35 at  $620\mu\mu$ , and at pH 7.0 at  $630\mu\mu$ . Thereafter the graph followed that of buffered haematin. With the human globulin-haematin mixture the displacement of the  $\alpha$  band towards the long-wave end of the spectrum was more gradual, being  $617\mu\mu$  at pH 8.2,  $619\mu\mu$  at pH 7.2,  $620\mu\mu$  at pH 6.4, and  $622\mu\mu$  at pH 5.8, after which it shifted more rapidly, being  $634\mu\mu$  at pH 5.1. Thereafter it shifted progressively with increasing acidity as shown in Fig. 1.

When alkaline haematin is added to human serum-albumin, the distinctive character of its spectrum, the increase in spectroscopic sensitivity, and its enhanced stability over a wide range of pH support the view that there is a chemical combination of haematin with serum-albumin, evidence for which has been already presented in the preceding section. It is evident that albumin is not merely acting as a protective colloid keeping haematin in suspension, since, on the addition of hydrochloric acid, the behaviour of the colloidal suspension of haematin protected from precipitation by gum arabic was so entirely different. Similarly, the behaviour of alkaline haematin in the presence of the egg albumin and human globulin mixture suggests that we are here dealing only with mixtures of alkaline haematin and these proteins. Heilmeyer (1933) noted that relatively large amounts of alkali were necessary to change the spectrum of haematin in the presence of serum to that of alkaline haematin, and the present findings indicate that increased amounts of hydrochloric acid are necessary to change the spectrum to that of acid haematin once the haematin-albumin compound, methaemalbumin, has formed.

#### *The Ultracentrifugal Investigation of Different Haematin-Albumin Mixtures*

The observations of Pedersen and Waldenström (1937) by the ultracentrifuge and electrophoresis on the bilirubin in blood and bile suggested that similar data might be of interest in regard to methaemalbumin. These authors, when studying both the directly reacting and indirectly reacting bilirubin derived from five patients with jaundice, had found that the sedimentation constants of the centrifuged bilirubin corresponded very closely with those of serum-albumin and haemoglobin, both of which have an approximately similar molecular weight. When a solution of serum-albumin, to which bilirubin had been added, was centrifuged, the greater part of the bilirubin was found to be associated with the serum-albumin, whereas in the presence of egg albumin this union did not take place, the bilirubin remain-

ing free. They concluded that both forms of bilirubin, namely that which couples directly with diazo-reagent and that which reacts only indirectly after the addition of alcohol, are bound to serum-albumin. Dr. A. S. McFarlane kindly investigated the behaviour of various albumin-haematin mixtures prepared for the purpose, which included serum-albumin derived from man, monkeys, and rabbits, as well as egg albumin.

*Human albumin fraction-haematin mixtures.* The albumin fraction was prepared from normal human serum and the ammonium sulphate completely removed by dialysis against running water and distilled water. To 10 c.c. of this albumin fraction, a solution of weakly alkaline haematin prepared from purified haemin, was added drop by drop until a satisfactory spectrum was obtained. This solution was subsequently dialysed against phosphate buffer solution at pH 8.0 and its behaviour investigated by the ultracentrifuge and cataphoresis tube. As in blackwater-fever serum, the haematin-albumin mixture (methaemalbumin) sedimented in the ultracentrifuge at the same rate as serum-albumin. The specimen of serum from which the albumin fraction was prepared contained only physiological amounts of bilirubin (0.2 van den Bergh units), so the problem presented in differentiating the bilirubin-albumin compound present in blackwater-fever serum did not arise. In the cataphoresis tube the human albumin-haematin mixture showed a homogeneous methaemalbumin boundary migrating at a slightly different rate from serum-albumin. There was present a small amount of unchanged serum. These facts were regarded as indicating a firm union of albumin and haematin which probably involved chemical linkage. Identical results were obtained with simian albumin-haematin mixtures investigated with the ultracentrifuge and will not be described.

*Egg-albumin-haematin mixture.* The egg albumin used in the experiment was prepared by Dr. R. A. Kekwick and had been purified by repeated recrystallization, dialysed against running water, distilled water, and finally against a buffer solution at pH 8.0. To this preparation slightly alkaline haematin was added until a good  $\alpha$  band was demonstrable spectroscopically ( $616\mu$ ); on reduction with sodium hydrosulphite a single broad-banded spectrum, similar to reduced haematin, was obtained. The haematin in this instance did not sediment with egg albumin in a completely homogeneous manner. On obtaining high speeds sufficient to precipitate egg albumin, 95 per cent. of the haematin did not come down with the albumin. A few per cent. might possibly have been associated with albumin, but the findings indicated a colloidal solution of haematin with all sized particles, some of which were smaller than egg albumin.

*Rabbit serum-haematin mixtures.* When rabbits were given intravenous injections of alkaline haematin (ferrie), prepared from pure haemin, in a dosage of 12 mg. per kilo bodyweight, all brown pigment disappeared from the plasma in a remarkably short time, within 30 min. of the injection. Spectroscopically, the pigment corresponded to alkaline haematin or colloidal haematin (ferrie) at a similar pH, the band being situated at  $616\mu$  to

617  $\mu\mu$  measured on the reversion spectroscopie. In man, receiving only 4 mg. per kilo, a brown discoloration of the plasma persisted for many hours, and methaemalbumin with its  $\alpha$  band at 623  $\mu\mu$  to 624  $\mu\mu$  was demonstrable spectroscopically in plasma collected 24 hours after the injection. Again, it was found that synthesized human methaemalbumin was practically all precipitated in the albumin fraction of human serum, only traces being removed with the globulins. When alkaline haematin (ferric) was added to rabbit serum, however, and the mixture half-saturated with ammonium sulphate, a considerable amount of brown pigment was found to come down with the globulin precipitate. This suggested that in the rabbit, haematin (ferric) was not so closely associated with serum-albumin as in man, while its rapid disappearance from the blood in rabbits was attributed to its being more rapidly eliminated in an uncombined state. In order to obtain additional data, rabbit serum-haematin mixtures and human serum-haematin mixtures (methaemalbumin) were examined by Dr. McFarlane, who compared their behaviour in the ultracentrifuge.

*Experiment 1.* A rabbit was given an intravenous injection of weak alkaline haematin (ferric), prepared from pure haemin, in a dosage of 12.5 mg. per kilo bodyweight; five minutes later, blood was collected and the serum subsequently separated. Spectroscopically, the pigment in the plasma corresponded to alkaline haematin (ferric). The brown pigment present in rabbit serum was found to sediment in the ultracentrifuge at the rate characteristic of serum-albumin. On half-saturation with ammonium sulphate, a brown globulin precipitate was obtained. This precipitate, when redissolved, gave a brown solution, the ultracentrifugal analysis of which showed 95 per cent. globulin and 5 per cent. albumin; all the colour, however, was associated with the albumin. There was thus a difference in the salt precipitability of the pigment in human serum and in rabbit serum. Owing to the rapidity with which haematin disappears in the rabbit, a large injection of haematin had been given and the blood had been collected shortly after the injection. It was thought that the amount of pigment precipitated with the rabbit globulin might possibly have been increased by the presence in the rabbit serum of some colloidal haematin which was carried down mechanically in the globulin precipitate, but the conclusion reached was that, as in man and monkeys, there was a firm union of haematin with part of the serum-albumin, while the globulin was unaffected.

*Experiment 2.* Two mg. of purified haemin dissolved in 1 c.c. of N/100 sodium hydroxide were added to 8 c.c. of human serum and to 8 c.c. of rabbit serum. On the Hartridge reversion spectroscopie the  $\alpha$  band in human serum was at 623  $\mu\mu$  and in the rabbit serum at 616  $\mu\mu$ , similar to that of alkaline haematin at a similar pH. On reduction with sodium hydrosulphite a two-banded spectrum resulted, the  $\alpha$  and  $\beta$  bands in the rabbit serum-haematin mixture being situated at about 561  $\mu\mu$  and 528  $\mu\mu$  and in human serum-haematin at 573  $\mu\mu$  and 528  $\mu\mu$ . The spectra, though not quite identical, were of similar pattern and differed from the single broad absorption band of reduced haematin obtained when alkaline haematin, colloidal haematin, or an egg albumin-haematin mixture is treated with sodium hydrosulphite. Similar spectra were also formed on treatment with sodium hydrosulphite and carbon monoxide.

*Comment.* In the ultracentrifuge the pigment in rabbit serum manifested exactly the same behaviour as in human serum; in both cases there was no reduction in colour as the centrifuge gained speed, while the whole of the brown colour sedimented at the same rate as the serum-albumin. This behaviour was in marked contrast to that of the egg albumin-haematin control in which there was a gradual reduction in depth of colour as the speed increased, and no evidence that any particular portion of the haematin sedimented at the rate of egg albumin. From these observations it would appear that, contrary to the writer's previous conclusions, haematin (ferric) does couple with rabbit serum-albumin and presumably other mammalian serum-albumins of rabbit type, but that the compound formed differs spectroscopically and in certain other respects from simian and human methaemalbumin. Perhaps examination of rabbit serum-haematin mixtures by spectrophotometric methods will reveal differences from the spectrum of alkaline or colloidal haematin which are not evident on ordinary direct spectroscopy or examination with the reversion spectroscope.

*The Production of Methaemalbumin (Pseudo-Methaemoglobin) in Man and Monkeys by Intravenous Injections of Alkaline Haematin (ferric)*

A considerable amount of literature exists on the subject of haematinaemia in man, and the condition has been described clinically by many workers including Schumm (1912), van den Bergh and Snapper (1915), Bingold (1930, 1932), and Duesberg (1933) in a variety of conditions such as pernicious anaemia, lead poisoning, gas gangrene infections, certain chemical poisonings, and acute yellow atrophy of the liver.

*Haematinaemia or methaemalbuminaemia.* Duesberg (1933) found that if a solution of haematin (ferric) prepared by dissolving 200 mg. of haemin in a 2 per cent. solution containing 0.8 per cent. of sodium hydroxide be injected intravenously into healthy men weighing 75 kilo., the haematin (ferric) could be demonstrated in the plasma regularly after 8 to 12 hours and frequently for 24 hours. Schumm's (1912) test for detecting haematin was employed. This was carried out by adding 1/10 volume of concentrated ammonium sulphide to the serum covered with a layer of ether, and examining spectroscopically through a depth of 4 cm. In the presence of haematin, a haemochromogen was formed with a prominent  $\alpha$  band at  $558 \mu\mu$ . Duesberg did not appear to have made any special spectroscopic examination of the serum from the viewpoint of the haematin spectrum, though Heilmeyer (1933) a year previously had investigated by spectrophotometry the absorption curves of alkaline haematin added to human serum. Heilmeyer found an absorption curve in the red with a maximum situated at  $620 \mu\mu$ , and an absorption in the green which presented a double-headed maximum at approximately  $535 \mu\mu$  and  $495 \mu\mu$ . He concluded, as a result of these and other observations, that alkaline haematin (ferric) when added to serum *in vitro* must combine with some protein constituent. No spectrophotometric or spectroscopic observations on the plasma from patients with so-called

haematinaemia were made by Heilmeyer and other workers on the subject, and the possible clinical significance of Heilmeyer's *in vitro* experiments with haematin and serum attracted no attention.

The observation reported by Fairley (1938) that alkaline haematin combined with human serum-albumin to form methaemalbumin *in vitro* implied that haematinaemia as such could not develop in man either after intravascular haemolysis or where there were large effusions of blood into cysts, joint cavities, or serous sacs. In order to determine whether, under experimental conditions, haematin persisted as such or formed methaemalbumin, solutions of alkaline haematin (ferric) prepared from pure haemin were injected intravenously into man in a dosage approximating to 4 mg. per kilo. of bodyweight. In all instances, on spectroscopic examination of the plasma, an  $\alpha$  band in the red appeared which, when examined on the Hartridge reversion spectroscope, was collinear with the  $\alpha$  band of methaemalbumin ( $623\ \mu\mu$ ) found in blackwater-fever plasma, and did not at all correspond spectroscopically with that of the original alkaline haematin ( $616\ \mu\mu$ ) injected into the patient. Details in regard to these haematin injections in man are recorded below.

*Intravenous injections of alkaline haematin in man. Case I.* An Indian seaman aged 40 years, who was convalescent after treatment for recent amoebic dysentery. He weighed 8 st. and was given an intravenous injection of 200 mg. of alkaline haematin (ferric) in 50 c.c. of normal saline, a dosage corresponding to 4 mg. per kilo. bodyweight. The haematin (ferric) solution was prepared by adding to pure haemin in saline just enough 10 per cent. sodium hydroxide to dissolve it completely. Prior to the injection, the plasma contained no methaemalbumin or haematin. In specimens of plasma collected one and two hours later methaemalbumin, not alkaline haematin, was demonstrable in a thickness of 1.3 cm. of plasma. Four hours later it had decreased somewhat, but was still evident spectroscopically in a thickness of 2.5 cm. Traces were detectable in this thickness up to 9 hours, but not after 12 hours, though, as subsequent results show, it would almost certainly have been found had thicker layers of plasma been examined. No significant increase in the bilirubin content of the plasma was found throughout the experiment, the amounts varying from 0.5 to 0.75 units (indirect reaction) in the six examinations made during the first 12 hours after the injection. Some 200 c.c. of blood were withdrawn after one hour, and 50 c.c. of serum obtained from it were used for separation of the albumin fraction which was found to contain the methaemalbumin as described later.

*Case II.* A man aged 60 years had been operated on for chronic cholecystitis and gall stones in the common duct. Cholecystectomy was performed by Mr. A. H. McIndoe, and two stones removed from the common bile duct, which was drained. At operation, the liver was noted to be firm and slightly nodular from mild biliary cirrhosis.

The patient was re-admitted to hospital for investigation 6½ months later, his weight then being 9 st. 7 lb. The indirect van den Bergh reaction was positive (1.75 units), direct reaction negative. The galactose tolerance test for liver function was normal. The urine contained no bile salts, bile pigments, or urobilin. An intravenous injection was given of 240 mg. of haemin in 100 c.c. of normal saline made sufficiently alkaline with 10 per

cent. sodium hydroxide to dissolve it completely; this was the equivalent of 4 mg. per kilo. bodyweight. Blood was collected at  $\frac{1}{2}$ , 2, and 4 hours after the injection. The plasma was brown and contained methaemalbumin in all specimens when examined in a layer of 5 cm. thickness. The bilirubin content of the blood remained unaltered (1.75 units). Some 50 c.c. of serum were collected for purposes of separation of the albumin fraction which was found to contain the methaemalbumin as described later.

*Case III.* A man aged 40 years suffered from Banti's disease with recurrent haematemesis, for which the spleen had been removed in 1929.

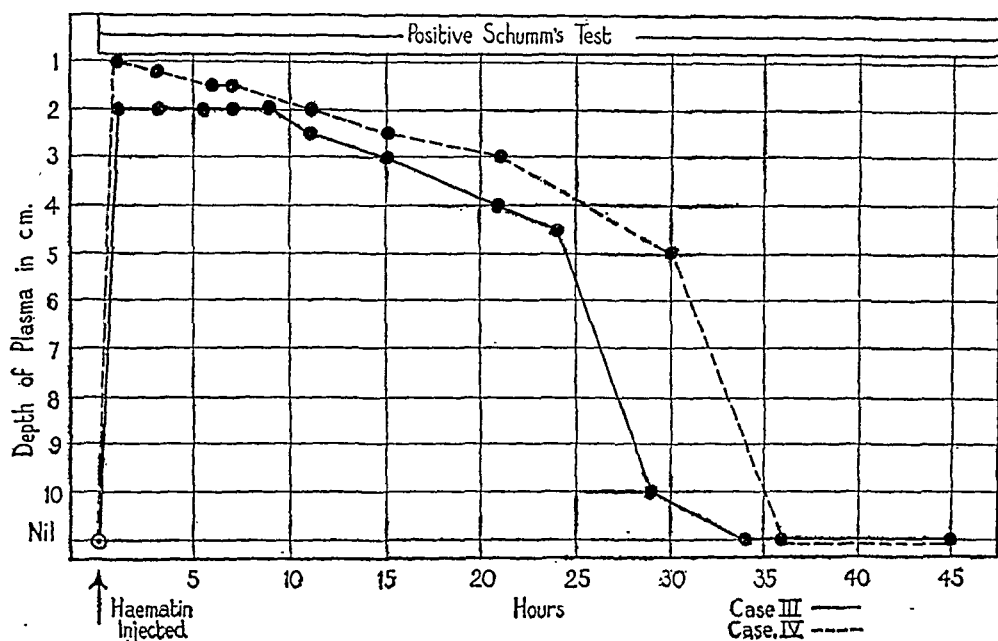


FIG. 2. The methaemalbumin concentration in the blood after intravenous injections of alkaline haematin (ferric)

In March 1938, a pyloroplasty was performed by Mr. A. H. McIndoe for a duodenal ulcer which was cauterized, while the coronary veins of the stomach were ligated to reduce the oesophageal varices. Examination of the liver at operation revealed early cirrhosis. A small piece excised for microscopic examination showed a fine unilobular fibrosis; the parenchyme cells contained considerable quantities of glycogen. Hepatic function appeared satisfactory judged by liver function tests. The patient received an intravenous injection of 300 mg. of haemin dissolved in 20 c.c. of 0.8 per cent. sodium carbonate, thus receiving approximately 4 mg. of alkaline haematin per kilo. bodyweight. The plasma showed no methaemalbumin before the injection, Schumm's test was negative, and the van den Bergh reaction equalled 0.8 units (indirect). One hour after injection, the  $\alpha$  band of methaemalbumin was visible spectroscopically in Harrison's apparatus in a depth of 2 cm. of plasma (Fig. 2), and this concentration was maintained for nine hours, after which it gradually decreased; it was still visible through a thickness of 4.5 cm. of plasma at the end of 24 hours, and through a thickness of 10 cm. of plasma at 29 hours, but after 34 hours it was no longer demonstrable spectroscopically. Schumm's test, which had been strongly positive throughout the period, remained positive for 45 hours

after the injection. The bilirubin, which equalled 0.8 units (indirect) before the injection, remained stationary for the next five hours and then actually dropped to less than 0.2 units, persisting at this value for 29 hours. It equalled 0.5 units at 34 hours and 1.0 units at 45 hours, a value approximating to the original level.

*Case IV.* A man aged 58 years, with a previous history of duodenal ulcer, was re-admitted to hospital for investigation. On this occasion no evidence of recurrence was obtained. A barium meal showed scarring due to healed ulceration, and there was no delay in gastric evacuation. Oral cholecystography showed a normally functioning gall bladder. On 5/5/39 he was given an intravenous injection of 200 mg. of haemin in 10 c.c of 0.8 per cent. sodium carbonate, thus receiving approximately 4 mg. of alkaline haematin per kilo. Prior to the injection the plasma contained no methaemalbumin, Schumm's test was negative, and the indirect van den Bergh was positive (0.2 units). One hour later the  $\alpha$  band of methaemalbumin was visible spectroscopically in Harrison's apparatus in a layer of plasma 1 cm. thick and Schumm's test was strongly positive. The concentration of methaemalbumin fell gradually and at 21 hours it was still visible in a layer of plasma 3 cm. thick. From the 24th to the 30th hour it was visible through 5 cm. of plasma, but it was no longer demonstrable at 36 hours, even in a layer of 11 cm. thickness. Schumm's test remained positive for 53 hours, but was not re-tested until 72 hours when it had become negative. The bilirubin remained stationary at 0.2 units for the first three hours and fell even below this value throughout the next 24 hours; no further observations were made.

*Rate of disappearance.* By the use of Harrison's (1938) apparatus it was found possible in Cases III and IV to make quantitative observations on the relative amount of pigment present at different times after the injection, based on the depth of plasma through which the  $\alpha$  band of the pigment was just visible spectroscopically. It will be seen that methaemalbumin was demonstrable spectroscopically in both cases in a layer of 2 cm. thickness for the first eight hours, through a layer of 4.5 cm. thickness for 24 hours, in a layer of 10 cm. and 5 cm. up to 29 and 30 hours respectively, and that it disappeared entirely in both cases somewhere between the 30th and 36th hour (Fig. 2). Though no longer visible spectroscopically, its presence or that of haematin was indicated by a positive Schumm's test persisting up to 45 and 53 hours in Case III and Case IV respectively. It should be noted that in Case IV, where the liver was normal, the methaemalbumin persisted longer in the circulation than in Case III, in which a mild hepatic cirrhosis had been found at operation. Delayed excretion in this case by the liver was not, however, anticipated as the other functional liver tests yielded no evidence of hepatic insufficiency. Though serial observations were made in all four cases by the van den Bergh reaction, no increase in the bilirubin content of the plasma was ever observed to follow the injection of alkaline haematin in man. These findings confirm those of Duesberg (1933) that the injection of haematin is not followed by any demonstrable increase in circulating bilirubin. They do not confirm his findings regarding experimentally



induced haematinaemia in man, inasmuch as the pigment present in the circulating blood was found to be methaemalbumin and not haematin. The evidence in support of this view is detailed below.

*The spectroscopic appearance and chemical behaviour of the pigment in plasma.*  
*Spectroscopic investigation.* On a Hartridge reversion spectroscope the  $\alpha$  band of the pigment in the red portion of the spectrum lay between 623 and 624  $\mu\mu$ , which is that of methaemalbumin, instead of at 616  $\mu\mu$ , which was the original position of the band of alkaline haematin (ferric) prepared by dissolving haemin in N/100 sodium hydroxide. It thus lay about midway between methaemoglobin (630  $\mu\mu$ ) on the one hand and sulphaemoglobin (618  $\mu\mu$ ) and alkaline haematin (616  $\mu\mu$ ) on the other. In many specimens, two other bands at 578  $\mu\mu$  and 540  $\mu\mu$  were visible, but these proved to be the  $\alpha$  and  $\beta$  bands of oxyhaemoglobin produced by haemolysis. It is this composite three-banded spectrum which in intravascular haemolysis and haemolytic anaemia has in the past so universally been mistaken for methaemoglobin by the clinical pathologist. When, as occasionally happened, the specimen of plasma contained no demonstrable bands of haemoglobin in thicknesses of 2 cm., only the  $\alpha$  band at 623 to 624  $\mu\mu$  and a general absorption in the green commencing about 545 to 548  $\mu\mu$ , were evident.

*Chemical reactions.* The chemical reactions used in the differentiation of methaemalbumin from the other pigments with an  $\alpha$  band in the red portion of the spectrum were carried out. In specimens of plasma from these cases, it was found that the  $\alpha$  band was not dispersed by 10 per cent. ammonium sulphide (1 drop per c.c.) or Stokes's reagent (2 drops per c.c.), whereas it was dispersed by concentrated ammonium sulphide (Analar) to form a haemochromogen with a prominent band at 558  $\mu\mu$  (Schumm's test). In specimens of plasma containing only traces of haemoglobin the colour changed from brown to red on reduction with sodium hydrosulphite, and a characteristic two-banded spectrum of haemalbumin (ferrous) with a prominent  $\alpha$  band at 572  $\mu\mu$  and a  $\beta$  band at 531  $\mu\mu$  was formed. A solution of haematin, similarly treated, gives the broad single-banded spectrum of reduced haematin (ferrous). The spectroscopic appearances and the chemical reactions described above show that the pigment in the plasma of these patients injected with haematin is identical with methaemalbumin occurring in the plasma of blackwater-fever patients, and differentiate it from methaemoglobin, sulphaemoglobin, and haematin. In order to ascertain whether the pigment was associated with albumin and to ensure haemoglobin-free samples for further chemical investigation, the serum-albumin fraction was studied in Cases I and II.

*The spectroscopic appearance and chemical behaviour of the pigment in the serum-albumin fraction.* *Preparation.* To 50 c.c. of the brown plasma was added an equal volume of saturated ammonium sulphate solution and the precipitated globulin was filtered off. The precipitate showed little colour and gave a weak positive Schumm's test, indicating that it contained only traces of haematin or methaemalbumin. The brown filtrate containing

the albumin fraction was next saturated with ammonium sulphate; the precipitate so obtained was strikingly brown, whereas the filtrate was water-clear. The precipitate was dissolved in distilled water and precipitated with ammonium sulphate, this process being repeated twice. Subsequently, the precipitate was dissolved in distilled water, dialysed in viscose sacs for several days in running water, and finally in distilled water until all traces of ammonium sulphate had disappeared.

*Spectroscopic appearance.* Examination on the reversion spectroscope showed an  $\alpha$  band at 623 to 624  $\mu\mu$ , and two faintly discernible  $\beta$  and  $\gamma$  bands in the green at approximately 540  $\mu\mu$  and 501  $\mu\mu$  respectively.

*Chemical reactions.* The reactions of the pigment in the albumin fraction to Stokes's reagent, 10 per cent. ammonium sulphide, sodium hydrosulphite, and concentrated ammonium sulphide, were identical with those already described. As the albumin fraction was haemoglobin-free the action of carbon monoxide after reduction with sodium hydrosulphite could be determined. The compound formed, carboxyhaemalbumin, closely resembled carboxyhaemoglobin spectroscopically, though it was not identical with it. Similarly, when the pigment in the albumin fraction was reduced with sodium hydrosulphite and a few drops of 10 per cent. sodium hydroxide were added, a haemochromogen (albumin-haemochromogen) formed with an  $\alpha$  band at 558  $\mu\mu$ , indistinguishable from globin-proto-haemochromogen. The behaviour of this pigment contained in the serum-albumin fraction derived from patients injected with haematin was similar in all respects to that obtained in the albumin fraction from patients with blackwater fever whose plasma contained methaemalbumin.

The results of these experiments, and other data to which space does not permit reference, indicate that in man when haematin (ferric) is injected experimentally into the blood stream or is formed in the circulation from katabolized haemoglobin liberated by intravascular haemolysis, it does not circulate as such, but combines immediately with serum-albumin to form methaemalbumin. It follows that all previous references in the literature to haematinaemia must be regarded as examples of methaemalbuminaemia. This also includes cases in which there is no spectroscopic evidence of methaemalbumin, for this pigment, as well as haematin, gives a positive Schumm's test. If plasma containing methaemalbumin which is demonstrable spectroscopically is diluted until no  $\alpha$  band is visible even in a depth of 10 cm. it will continue to give a positive Schumm's test. Similarly, after injection of haematin in man, the plasma continued to yield a positive Schumm's test for many hours after methaemalbumin was no longer demonstrable spectroscopically.

*Injections of haematin (ferric) and reduced haematin (ferrous) into monkeys.* *Haematin (ferric).* In monkeys (*Macacus rhesus*) when alkaline haematin (ferric) prepared from pure haemin was introduced into the blood-stream by intracardiac injections in a dosage of 12.5 to 20 mg. per kilo. bodyweight, methaemalbumin was at once formed. The  $\alpha$  band at 623 $\mu\mu$  was demon-

strable in the serum for six hours or longer, and no evidence of the original haematin with an  $\alpha$  band at  $616\mu\mu$  was forthcoming. The various chemical reactions described for human methaemalbumin were also found to be characteristic of simian methaemalbumin, and call for no further comment. One experiment is of special interest as the behaviour of the plasma pigment in the ultracentrifuge was studied by Dr. A. S. McFarlane. A small monkey, *Macacus rhesus*, received 15 mg. per kilo. of alkaline haematin, prepared from purified haemin, the injection being given by the intracardiac route. Blood was collected by ventricular puncture 15 to 20 min. later. The spectrum resembled that of methaemalbumin and the  $\alpha$  band was collinear with that of human methaemalbumin ( $623\mu\mu$ ). Dr. McFarlane reported that the brown pigment in the monkey serum sedimented in the ultracentrifuge at the rate characteristic of serum-albumin. Visual observation of the sedimentation picture also showed that no colour was associated with the globulin component of this serum, and half-saturation of the serum with ammonium sulphate gave a practically colourless globulin precipitate.

*Reduced haematin (ferrous).* When reduced alkaline haematin (ferrous), prepared from alkaline haematin (ferric) by reduction with sodium hydrosulphite, was slowly injected into the ventricular cavity, methaemalbumin was at once produced, the reduced haematin (ferrous) being oxidized to haematin (ferric) in the blood-stream. Methaemalbumin was found to be concentrated in the albumin fraction of the plasma of monkeys injected with either haematin or reduced haematin, and was found to be identical in regard to chemical tests and spectroscopic appearance with that occurring in man. Sodium hydrosulphite proved a dangerous substance to introduce into the circulation and could not be given to man. Experiments *in vitro* with human plasma revealed that when reduced haematin in the absence of excess of sodium hydrosulphite was added to plasma, methaemalbumin was formed, the reduced haematin (ferrous) being oxidized to haematin (ferric) which united with serum-albumin to form methaemalbumin.

These experiments with reduced haematin (ferrous) have a possible bearing on the mode of extracorporeal haemoglobin disintegration in intravascular haemolysis in man. Since methaemoglobin is not spectroscopically demonstrable in the plasma in intravascular haemolysis, such as blackwater fever, nocturnal haemoglobinuria, &c., it is probably not formed as an intermediary stage in extracorporeal haemoglobin katabolism. The more likely route is that the haemoglobin is split in the circulation into globin and reduced haematin (ferrous); the latter is immediately oxidized to haematin (ferric) which combines with serum-albumin to form methaemalbumin.

#### *Summary and Conclusions*

1. Methaemalbumin (pseudo-methaemoglobin) is immediately produced by the addition of alkaline haematin (ferric), prepared from pure haemin, to human and simian plasma at  $37^{\circ}\text{C.}$ , but not to the plasma of other mammals.

2. Methaemalbumin is also formed when alkaline haematin (ferric) is added to the albumin fraction, but not to the euglobulin and pseudo-globulin fractions, of human and simian plasma.

3. When alkaline haematin (ferric) is added to the individual proteins contained in the albumin fraction of human serum, crystalalbumin, seroglycoid, and globoglycoid, methaemalbumin is formed with crystalalbumin only.

4. On the reversion spectroscopy synthesized methaemalbumin presents a three-banded spectrum composed of an  $\alpha$  band situated at  $623\mu\mu$  to  $624\mu\mu$ , a  $\beta$  band at  $540\mu\mu$  to  $541\mu\mu$ , and a  $\gamma$  band at  $500\mu\mu$  to  $501\mu\mu$ , superimposed on a general absorption; this is identical with the spectrum observed when methaemalbumin is concentrated in the albumin fraction of blackwater-fever plasma.

5. The chemical reactions of synthesized methaemalbumin are identical with those observed with methaemalbumin found either in blackwater-fever plasma or concentrated in its albumin fraction.

6. On reduction with sodium hydrosulphite, a compound, haemalbumin, with a two-banded spectrum is formed containing ferrous iron; further treatment with carbon monoxide leads to the formation of carboxy-haemalbumin, characterized by a spectrum very similar to that of carboxy-haemoglobin.

7. Haemalbumin differs from haemoglobin in not combining loosely with oxygen; this may be due either to an absence of polymerization or to its possessing a different protein component, albumin instead of globin.

8. The effect of changes in pH on the wave length of the  $\alpha$  band in various haematin mixtures was investigated.

9. An enhanced stability in the presence of acid was demonstrated with human serum-haematin and human serum-albumin-fraction-haematin mixtures compared with colloidal haematin, recrystallized egg-albumin-haematin, and the globulin-fraction-haematin mixtures.

10. A similar enhanced stability in the presence of alkali is known to occur; these data indicate that human serum-albumin is not merely acting as a protective colloid keeping haematin in solution, but is chemically combined with haematin.

11. When human albumin-haematin mixtures are examined in the ultracentrifuge the pigment sediments at the same rate as serum-albumin, whereas in an egg albumin-haematin mixture it sediments in a heterogeneous manner like colloidal haematin. These facts also indicate a firm union of albumin and haematin probably involving a chemical linkage.

12. The conclusion reached from this study is that methaemalbumin is a definite chemical compound consisting of a prosthetic group, oxidized haematin (ferric), and a protein component, native serum-albumin.

13. In rabbit serum-haematin mixture the spectrum resembles that of alkaline haematin, not methaemalbumin; on the ultracentrifuge, however, the pigment sediments at the same rate as serum-albumin so that here, also, an albumin-haematin compound is probably formed.

14. On injecting alkaline haematin (ferric) intravenously in man in a dosage of 4 mg. per kilo, methaemalbumin and not haematin was found in the plasma, which was brown coloured.

15. Methaemalbumin was concentrated in the albumin fraction of such sera, and was found to differ from alkaline haematin both spectroscopically and in its chemical behaviour.

16. In both the plasma and the albumin fraction of the serum the  $\alpha$  band was situated at  $623 \mu\mu$ , whereas with the original alkaline haematin it was at  $616 \mu\mu$ . Other differences were also demonstrable.

17. On the addition of sodium hydrosulphite a double-banded spectrum with an  $\alpha$  band at  $572 \mu\mu$  and a  $\beta$  band at  $531 \mu\mu$  was found, owing to the formation of haemalbumin. In the case of alkaline haematin a broad single-banded spectrum is produced under similar conditions.

18. Other differences include the stability of methaemalbumin in the presence of alkalis and the formation of carboxyhaemalbumin after treatment with sodium hydrosulphite and carbon monoxide.

19. Similar findings are recorded with monkeys of the species *Cercopithecus aethiops*, *Macacus rhesus*, and *Macacus sinicus*.

20. In addition, it was found that when reduced alkaline haematin (ferrous) was injected intravenously in these animals, methaemalbumin was formed, indicating that in the blood-stream reduced haematin (ferrous) was immediately oxidized to haematin (ferric) which combined with serum-albumin to form methaemalbumin.

21. The bearing of these findings on the katabolism of circulating extra-corpuseular haemoglobin in intravascular haemolysis is discussed.

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BRONCHIAL ADENOMA<sup>1</sup>

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With Plates 8 to 12

*Introduction*

THE first description of this type of tumour was given by Mueller in 1882. At a necropsy on a young woman who had suffered from a cough and foetid blood-stained sputum for eight years, he found a benign pedunculated adenoma partially occluding the left main bronchus. Numerous bronchiectatic cavities were present distal to the tumour, and histologically the growth consisted of proliferated mucous glands. Confusion with bronchial carcinoma followed this isolated example, a confusion which unfortunately still exists. The benign nature of bronchial adenoma was not recognized again until 1927 when Heine found a polypoid tumour the size of a grape in the right middle lobe bronchus of a woman 45 years of age. It had given rise to no symptoms during life, although bronchiectasis was found distal to the tumour. Heine thought that the growth was an adenoma arising from the bronchial mucous glands; the cells were well differentiated and showed no unruly growth. The following year, Reisner (1928) reported the death of a woman from a putrid empyema secondary to an obstructing bronchial polyp which proved to be an adenoma. It is worth noting that these first three examples of bronchial adenomata were all discovered at necropsy.

The first examples to be diagnosed during life, which were also treated successfully, were described by Kramer in 1930. He drew attention to the long duration of symptoms and to the benign course in these patients, although he suggested that local malignancy might occur. His treatment consisted of bronchoscopic removal, the application of diathermy, and the insertion of radon seeds. This paper led to a more general recognition of these growths, and Wessler and Rabin in 1932, writing on benign tumours of the bronchus, described 17 examples of which 12 were adenomata. These authors were also the first to give a comprehensive account of the clinical features, with emphasis on the mechanical effects of the tumour and the value of early bronchoscopy. They thought that a cure could usually be effected by simple removal in the early stages.

Morlock and Pinchin (1935) found 6 per cent. of benign tumours in a series of 150 bronchial new growths investigated bronchoscopically. In the same year Kramer and Som (1935) found 23 adenomata in a series of 355 cases of

<sup>1</sup> Received December 25, 1940.

bronchial tumour (6 per cent.). Since then recorded examples have become increasingly numerous, and in 1936 Gerlings was able to collect no fewer than 34 from the literature of the previous four years, whereas Patterson (1930) could find only three. Pollack, Cohen, and Gnassi (1938) found 51 adenomata in a series of 104 benign bronchial neoplasms collected from published records. Other examples of this condition have been described by Fried (1934), Hart (1937), Mallory (1938), Peterson (1936), Rosenblum and Klein (1935), Tchertkoff and Klosk (1939), and Zamora and Schuster (1937). Of the more recent contributions that by Jackson and Konzelmann (1937) is the most important. These authors showed that the pathology of bronchial adenoma was still obscure and that the tumour did not always have a highly developed glandular structure. Variations in structure have not hitherto been fully described and have given rise to considerable difficulty in diagnosis. Womack and Graham (1938) advanced the theory that bronchial adenomata were developmental in origin, analogous to mixed salivary gland tumours; Brock (1938) and Price Thomas (1940) have also written of this resemblance to salivary tumours.

### *Clinical Features*

About 100 convincing examples of bronchial adenoma have been recorded. Twenty-two others, which have been seen at the Brompton Hospital, are described here and are incorporated with a survey of the literature. Unfortunately, published records often failed to mention some important point, and such cases have had to be excluded from each particular analysis. The number available for analysis in each instance has therefore been recorded in the tables. In addition, relevant facts about those examples which have been reported in the available literature are given in Appendix I. The Brompton Hospital series is summarized in Appendix II.

*Sex and age incidence.* Adenoma of the bronchus is as common in females as in males. Of the Brompton Hospital patients, 12 were men and 10 women, and adding these to the recorded cases, it was found that 62 per cent. of the total occurred in females. Seventy per cent. give rise to symptoms in the third and fourth decades of life, and a majority are recognized between the ages of 30 and 40 years. The average age at the onset of symptoms was 28 years, with no difference between the sexes.

*Aetiology.* The aetiology of glandular tumours in general is obscure and nothing is known of the causation of adenoma of the bronchus. Occupation does not seem to be a factor; no recorded case could be found in which the occupation was mentioned, and in the Brompton Hospital series a relationship with employment was not apparent. Another possible factor, previous disease of the lungs, appears to be excluded, for it has not figured in the literature nor been found in the Brompton Hospital series. Peroni (1930) failed to cause tumour formation by experimental irritation of the bronchial wall in dogs. More recent animal experiments by Campbell (1937) have shown that bronchial tumours, including adenomata, may be produced in mice by means of carcinogenic substances, but deductions from such results are



applicable to man only with reservations, since animals vary in their susceptibility, and mice are prone to develop spontaneous pulmonary cancers. Jackson and Konzelmann (1937) had thought that bronchial adenomata might be associated with nerve elements and related to the carcinoid tumours of the gastro-intestinal tract, but they found that these adenomata failed to show the argentophile properties of carcinoids.

*Duration.* A cardinal feature of an adenoma of the bronchus, and one distinguishing it from bronchial carcinoma, is the long history of symptoms.

TABLE I  
*Analysis of Clinical Features*

	Cases available for analysis	Primary		Secondary						
		Haemoptysis	Cough	Dyspnoea	Atelectasis	Pleurisy and pneumonia	Pyrexial attacks	Bronchiectasis	Empyema	Lung abscess
Examples from the literature	68	53								
	47		35	13						
	70				52	25	13	11	6	1
Brompton Hospital series	22	20	18	8	20	10	7	4	1	1
Total	90	73								
	69		53	21						
	92				72	35	20	15	7	2
Percentage		81	77	30	78	38	22	26		

The longest recorded duration is 40 years (Kernan 1935), and one patient in the present series (No. 19) had symptoms for 23 years. The average duration in all patients was five years prior to the time of diagnosis, and no fewer than 87 per cent. had had symptoms for a year or more.

*Symptoms.* Bronchial adenomata cause two distinct groups of symptoms, those which are directly due to the tumour and those which result from bronchial obstruction, followed after an interval by infection of the lung. The two principal early symptoms are haemoptysis and cough. Haemoptysis is the more prominent and was present in 81 per cent. of all cases (Table 1). Bleeding is usually recurrent with periods of intermission, and as it is often not profuse and the blood is promptly expelled, the haemoptysis tends to start and end abruptly. The cause is surface ulceration of the tumour, with bleeding from a vascular connective tissue layer just below the epithelium. Wessler and Rabin (1932) noted attacks of haemoptysis in women related to menstruation, and this finding has also been reported by Kramer (1930), Gerlings (1936), and Jackson and Konzelmann (1937). In one of the Brompton Hospital patients (No. 19) haemoptysis often started immediately after a menstrual period. As a general rule the amount of bleeding is small, up to a few ounces, but sometimes very large haemoptyses occur, as in two patients (Nos. 2 and 17) one of whom lost as much as two pints of blood on one occasion. Haemoptysis may be the only symptom, especially in the initial stages

of the disease. Its recurrent character is illustrated by another Brompton Hospital patient (No. 15) whose only complaint was attacks of haemoptysis which had occurred yearly for nine years; a small bronchial adenoma was found, which was not obstructing the airway.

Cough is an almost constant feature and was prominent in 78 per cent. of patients. At first dry and irritating, it becomes productive only with the onset of infection.

The second group of symptoms is caused by bronchial obstruction producing collapse of that part of the lung distal to the tumour. This process is usually gradual, but occasionally a large bronchus may suddenly become occluded causing acute dyspnoea and distress (Kernan, 1935). One patient (No. 9) developed an acute absorption collapse of the lung after an attempt to remove a bronchial adenoma bronchoscopically. Bronchoscopy was immediately undertaken again, and after the bronchus had been cleared of blood-clot and debris the lung rapidly re-expanded.

Dyspnoea caused by a bronchial adenoma may take several forms. So-called 'asthmatoïd' attacks have often been reported, in which the patients complain of paroxysms of shortness of breath and wheezing, even accompanied by expiratory bronchial spasm like true asthma. Dyspnoea was a prominent symptom in 31 per cent. of all cases, and in about 10 per cent. was paroxysmal. Another type of dyspnoea is that due to partial or complete collapse of the lung secondary to an obstructing tumour. This is usually increased by exertion, may be progressive, and is sometimes recurrent (Ashbury, 1929; Kernan, 1935).

Infection produces sputum, pain from associated pleurisy, and attacks of pyrexia, and in over 60 per cent. of cases eventually leads to septic bronchiectasis, lung abscess, or empyema. Recurrent pyrexial attacks occurring over a period of several years are a distinctive feature of a benign bronchial tumour, and were present in 20 per cent. of all cases. A history of pleurisy or pneumonia in the region of the tumour is also very common; it was found in over one-third of the patients and was frequently recurrent.

*Physical examination.* The general condition of a patient suffering from bronchial adenoma is usually good, for toxæmia is not evident until the later stages. Then clubbing of the fingers is also often seen in those with chronic infection. Physical examination of the chest reveals no abnormality until the onset of complications. Then the signs are those of bronchial obstruction and collapse of the lung, with or without infection. Collapse is due to bronchial occlusion, after which the air in the affected site is absorbed. This collapse may be intermittent, and Zamora and Schuster (1937) have suggested as an explanation that bronchial adenomata, being vascular tumours, may vary in size. More probably, recurrent attacks of collapse are due to plugging of an attenuated airway by viscid sputum which is later expectorated. The extent of the collapse depends upon the situation of the tumour. If a main bronchus is occluded, collapse of the whole lung follows, and this was seen in three of the Brompton Hospital patients (Nos. 1, 9, and 16). More usually the tumour arises in a lobar bronchus and causes collapse of that lobe only.

Obstructive emphysema has been mentioned as a feature (Wessler and Rabin, 1932; Jackson and Jackson, 1932; Peterson, 1936; Clerf and Crawford, 1936), but it was not observed in the Brompton Hospital series. Jackson (1917) described a valvular type of obstruction in such cases whereby air enters a lobe, but cannot be expelled from it. In the later stages, after the onset of infection, physical signs become more prominent. Such complications as bronchiectasis, empyema, or lung abscess give rise to their special clinical features, and it is not unusual for these to be recognized and treated with the causal tumour remaining undetected.

*Radiological and bronchoscopic examination.* Simple radiography of the chest shows no abnormality in the early stages, unless the tumour is already large enough to show as an opacity in the lung. Such a tumour usually appears as a rounded, sharply defined, homogeneous shadow, frequently with an area of collapse distal to it (Plate 8, Fig. 3). After a bronchus has become obstructed, there is radiological evidence of collapse; this is patchy at first and sometimes intermittent, but later it commonly involves an entire lobe or more. A lateral view is important for localizing an affected area.

Bronchography may reveal a filling defect (Plate 8, Fig. 4) or occlusion of a bronchus. The shadow of the blocked bronchus is usually nipped off sharply owing to the pool of oil lying in a gutter round the growth (Plate 9, Fig. 5); sometimes it may show a cup-like appearance, but the 'rat-tailed' termination so common with a constricting carcinoma is never seen. After an adenoma has been removed, bronchography can be used to determine the form of the bronchi in the lobe which was previously collapsed. Lloyd (1931) described a benign bronchial tumour with a ball-valve action; iodized oil in the bronchus was seen on radioscopy to accumulate at the site of the growth until on extreme inspiration it suddenly dropped past the obstruction into the lower lobe. When an extrabronchial portion of the growth is suspected, a tomogram may help to outline its size.

Histological examination of tissue removed through the bronchoscope is the only method of establishing the diagnosis of bronchial adenoma. There is no widening of the main carina, since the mediastinal glands are not enlarged. The tumour itself is a pinkish-white, vascular body which may be sessile or pedunculated, and bleeds readily on touching; haemorrhage is often so free that it makes removal difficult or impossible. There is no infiltration of the surrounding structures and the bronchus is therefore freely movable. Pus may be seen oozing up from an infected area distal to the growth, and this also tends to obscure the view. It is rarely possible to decide by bronchoscopy whether the tumour extends outside the bronchus.

*Prognosis.* The prognosis of a bronchial adenoma depends mainly upon its early recognition. It has been shown that sometimes years may pass without troublesome symptoms, but usually the course is progressive and most patients develop secondary infection of the lung unless they are treated. Of the 22 Brompton Hospital patients, 15 are still living and seven are dead. The average duration of the disease since its onset in those who are still alive is

10 years, the longest being 24 years (No. 19). The average duration in those who died was eight years. Sometimes an adenoma may remain stationary for an indefinite period, as in one patient who was observed for many years by Clerf and Crawford (1936). Death may result from haemoptysis, although this is rare in untreated cases. Fatal haemoptysis has usually occurred after attempts to remove the tumour through the bronchoscope, and especially after the use of surgical diathermy.

When the condition is recognized and treated before bronchial obstruction and secondary pulmonary infection have occurred, the prognosis is

TABLE II  
*Collected Results*

	Cases available for analysis	Clinical course				Cause of death				
		Cured	Improved	Stationary	Died	Empyema and lung abscess	Unassociated disease	Haemoptysis	Following thoracotomy	Pneumonia
Examples from the literature	42	21	8	3	10	4	3	0	2	1
Brompton Hospital series	22	5	9	1	7	2	1	3	1	0
Total	64	26	17	4	17	6	4	3	3	1
Percentage		74			26					

good, but once these complications have arisen the risks are far greater and the prospect of a cure becomes correspondingly remote. Even if the tumour is removable at this stage, the underlying condition in the lung still remains and continues to dominate the prognosis. Seventeen deaths from bronchial adenoma have been recorded (Table II), and 10 of these were due to pulmonary infection. In five of the infected cases death occurred after the tumour had been removed, in three it was due to operative treatment, and the remaining two had not been treated. Although many authors have mentioned the possibility that a bronchial adenoma may become malignant, no case has yet been recorded where death has occurred from this cause. Kramer and Som (1935) pointed out that an adenoma which extended outside the bronchus had a worse prognosis than one which was confined to the lumen. This is because the extrabronchial portion cannot be removed through the bronchoscope, and consequently the added risk of a major surgical procedure has to be faced.

*Diagnosis.* In the diagnosis of bronchial adenoma the problem is almost always that of recurrent haemoptysis and its causation, and the published records show that there are two common errors in diagnosis, namely the confusion of this condition with pulmonary tuberculosis and bronchial carcinoma. Features such as haemoptysis, infection of the lung, and a radiological opacity particularly suggest pulmonary tuberculosis, a diagnosis which was actually made in three instances in the present series. One

patient (no. 17) complaining of cough with haemoptysis was at first thought to have a lung abscess and later phthisis, for which he was then treated over 11 years. Much of this time was spent in sanatoria where he continued to have large haemoptyses; an attempt at artificial pneumothorax failed and later phrenic evulsion was done. Eventually the bronchial tumour was discovered and thought to be a carcinoma; lobectomy was attempted unsuccessfully, and as a last resort the growth was excised through the bronchoscope in order to clear the airway. Seven years later the patient is still alive and well, although requiring occasional bronchoscopy to remove remains of the tumour, which is now recognized to be a bronchial adenoma. In another case (no. 12) the onset was gradual, with cough and sputum, haemoptysis, lassitude, and intermittent fever. Pulmonary tuberculosis was diagnosed and the patient sent to a sanatorium where an artificial pneumothorax was induced. Two years later, investigation revealed a bronchial tumour which then was thought to be malignant. Lobectomy was attempted, but was impracticable, and after a course of deep X-ray therapy, the patient was sent home to await his death. Four years later he was still alive and well, although not completely free from symptoms. The third patient (no. 3) was also sent to a sanatorium for three months before a correct diagnosis was made. These instances are cited to illustrate how easily a bronchial adenoma may be overlooked. It is among such examples of haemoptysis in patients with a course atypical of pulmonary tuberculosis, with persistent absence of tubercle bacilli from the sputum, and with unusual radiological features, that these tumours will be found by the bronchoscope.

The frequent occurrence in patients with bronchial adenoma of the combination of haemoptysis, bronchial occlusion, and pulmonary collapse most often leads to a diagnosis of carcinoma. This is in fact the commonest error, and no fewer than 13 of the 22 cases forming the present series were so regarded at one time. Adenoma of the bronchus is as common in females as in males and is most often recognized between the ages of 30 and 40 years; in contrast, bronchial carcinoma has its greatest incidence in the fifth and sixth decades of life and occurs four times more frequently in males than in females (Davidson, 1930). A survey of the 428 proved instances of bronchial carcinoma which have been seen at the Brompton Hospital during the past 10 years showed that only one-eighth occurred in women, and that, on an average, they gave rise to symptoms 10 years later than did the adenomata. Again, the duration of symptoms from a carcinoma is usually a matter of months, while from an adenoma it frequently runs into years. Rarely a large bronchial adenoma, appearing as a dense, rounded opacity in the lung on X-ray examination, may be regarded as a localized carcinoma or a single metastatic growth. Examination of tumour tissue removed through the bronchoscope is the only certain method of distinguishing between the two conditions, but when there is difficulty in histological diagnosis, as for instance with a small or distorted specimen, the history and clinical findings may have to be the deciding factor.

Other sources of error are pneumonia or pleurisy occurring in the collapsed lung, since these may be regarded as independent conditions and the causal tumour may thus pass unrecognized. Similarly bronchiectasis or a collapsed lobe may simulate a simple unresolved pneumonia, although the distinction can almost always be made by bronchography. Empyema or lung abscess may also be regarded as primary conditions when a bronchial adenoma is responsible. One patient in this series (No. 1) had a chronic empyema apparently as a sequel to pneumonia, and this was drained surgically; a persistent sinus developed followed by large haemoptyses, but it was not until a year later that the underlying adenoma was discovered.

### *Pathology*

*Distribution.* A bronchial adenoma arises invariably in one of the larger bronchi and there is no record of this tumour occurring in the periphery of the lung. It is twice as common on the right side as on the left, and 30 per cent. originate in the right lower lobe bronchus. Fig. 1 shows the distribution of 70 bronchial adenomata, comprising 22 from the Brompton Hospital and 48 from published records.

*Morbid anatomy.* The appearance of the tumour as seen through a bronchoscope has already been described. As the growth enlarges it becomes elongated owing to its confinement within the lumen of the bronchus and it is forced upwards towards the trachea by coughing and expiratory efforts (Plate 9, Fig. 6). Wessler and Rabin (1932) noted that it often forces a way between the bronchial cartilages and expands again outside the bronchus, thus forming a mass shaped like a dumb-bell or cottage loaf (Fig. 2). The extra-bronchial portion may attain a very large size, compressing but not invading the surrounding lung (Plate 10, Fig. 7). Kramer and Som (1935) thought that this variety of adenoma originated in the glands which lie deep in the bronchial wall between the cartilages, and in consequence they named it the 'intramural' type.

*Histology.* A bronchial adenoma is covered by ciliated columnar epithelium which rests upon an intact basement membrane and is continuous with that lining the bronchus. The tumour cells do not invade the epithelium, showing that growth is expansive rather than infiltrative. It is common to find the epithelium covering these tumours either partly or completely stratified as a result of chronic irritation. Beneath this epithelium there is a deep layer of loose connective tissue containing numerous thin-walled blood-vessels. In cases of some duration the surface of the growth is often ulcerated and there is an inflammatory infiltration of its outer layers.

The tumour proper consists of masses of cells supported by a scanty stroma of vascular fibrous tissue. The cells of the tumour are characteristic; they are most commonly cuboidal in shape, although those forming the walls of alveoli or tubules may be columnar or flattened, the cytoplasm is scanty and clear, forming a fringe around the large nucleus, and vacuolation of the cytoplasm is seen occasionally. The nuclei are large, uniform in size, and

round or oval in shape, or they may be elongated or fusiform in areas where the cells have become flattened, but they are rarely irregular. There is a delicate nuclear membrane and a fine granular network of chromatin; nucleoli are seldom seen. Mitoses are rare and so is true nuclear hyper-

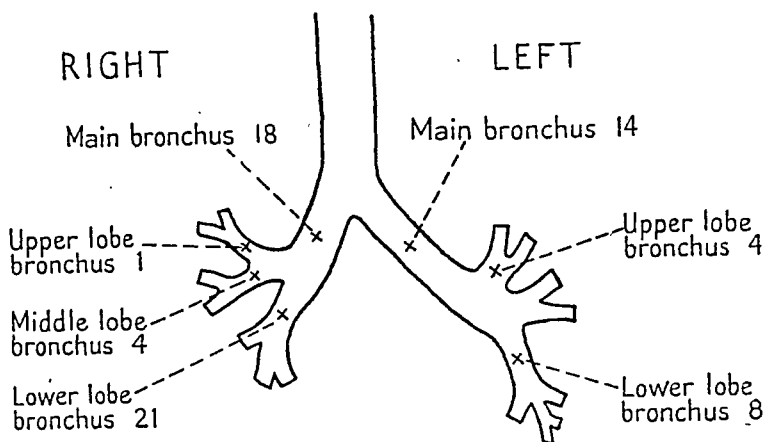


FIG. 1

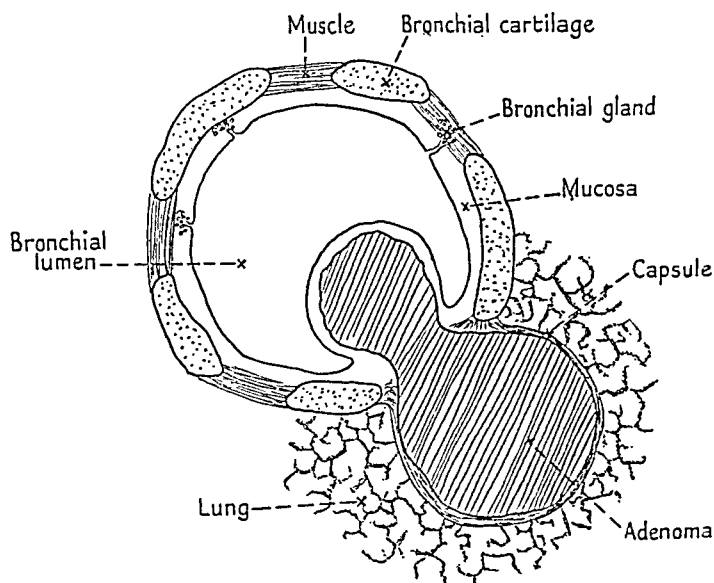


FIG. 2. Diagram of bronchial adenoma with extrabronchial extension

chromatism, but it is not unusual in bronchoscopic specimens to find degenerative and pyknotic changes in the nuclei, making them appear hyperchromatic. The tumour cells always show a regular arrangement, varying somewhat in individual cases. At the periphery the cells tend to stain more darkly and are scattered irregularly in columns and masses at the margin of the connective tissue layer. This appearance is exaggerated in bronchoscopic specimens, owing to the effects of trauma and infection, and may be mistaken for malignancy. The cells of the tumour proper are always

epithelial in type and usually show some differentiation along glandular lines. The arrangement varies from highly specialized alveoli and tubules in some examples to the aggregation of cells in solid acini and anastomosing columns or the formation of irregular parenchymatous masses in others. Occasionally the glandular spaces contain mucus. The scanty fibrous stroma is supplied with numerous blood-vessels. In two examples in the present series (Nos. 8 and 16) the cell masses were widely separated by a loose hyalinized connective tissue network which was rich in fibroblasts; Jackson and Konzelmann (1937) reported a similar finding. Womack and Graham (1938) described areas of bone formation in an adenoma of the bronchus, which must be

TABLE III  
*Histological Classification*

	Group 1	Group 2
Recorded cases	11	17
Brompton Hospital series	6	16
Total	17	33

excessively rare, since no other instance is recorded and it was not observed in the Brompton Hospital series.

The most striking histological characteristic of bronchial adenomata is the uniformity of their cells both in size, shape, and staining properties, together with the absence of any sign of unruly growth. Distant metastases have not so far been recorded, nor were any found in the six cases that came to necropsy at the Brompton Hospital (Nos. 2, 6, 8, 10, 14, and 21), although the average duration of the disease in these patients was eight years. In four (Nos. 2, 8, 14, and 21) sections were taken from the regional lymph nodes, and showed no sign of growth. Although the type of cell is essentially the same in all bronchial adenomata, there are distinct variations in the arrangement of the cells which enable these tumours to be classified into two histological groups. Group 1 includes those examples which have a highly differentiated structure consisting of tubules and glandular alveoli. These tumours are typically adenomatous and were the first to be recognized, so that the early examples in the literature were all of this type. In the last few years it has become apparent that there are other tumours which behave like these typical adenomata both clinically and pathologically. They differ from the highly specialized variety only in the fact that the arrangement of their cells includes both glandular and less differentiated epithelial formations. Group 2 comprises these tumours which have an atypical structure and show a lesser degree of glandular formation, together with solid acini and irregular masses of cells. Owing to the lack of a detailed histological description in many instances, only 28 examples were found in the literature which could be classified; together with the 22 Brompton Hospital cases they formed a total of 50, and their distribution is shown in Table III. It will be seen that more than 60 per cent. of these tumours had an atypical structure and only 17 out of 50 were of the highly specialized type previously



regarded as typical. Nevertheless, follow-up observations have shown that the atypical forms pursue the same benign course as those which have a highly differentiated glandular structure.

*Histological diagnosis.* The variations in structure which occur in bronchial adenomata have caused confusion in the past because failure to recognize them has falsified diagnosis. Earlier writers laid so much stress on the

TABLE IV

*Results According to Histological Structure*

	Recorded cases		Brompton Hospital series		Total	
	Number	Result	Number	Result	Number	Result
<i>Group 1</i>	10		6		16	
Cured		4		1		5
Improved		1		2		3
Stationary		1		1		2
Died		4*		2†		6
<i>Group 2</i>	16		16		32	
Cured		9		5		14
Improved		4		6		10
Stationary		1		0		1
Died		2‡		5§		7

\* 3 from unassociated disease, 1 from lung suppuration.

† Both from haemoptysis.

‡ 1 after pneumonectomy, 1 after lobectomy after four years' treatment.

§ 2 from haemoptysis, 1 from unassociated disease, 1 after thoracotomy, 1 from lung abscess.

Average length of follow-up—four years.

glandular arrangement of the cells that the tendency has been to regard all tumours without this structure as carcinomatous. Thus Krelinger (1913) described a so-called 'cylindrical cell carcinoma' of the left main bronchus which was polypoid and produced symptoms over a period of six years, and Malkwitz (1921), von Pein (1929), and Negus (1933) recorded similar examples. Lloyd (1931) described a bronchial polyp occurring in a woman of 28 years and causing haemoptysis; the growth, which was thought to be malignant, was coughed up and on section showed only glandular hypertrophy. Similarly Clerf and Crawford (1933) and Moersch and Bowring (1935) both reported patients who had lived for several years after local treatment for growths showing histological features suggestive of adenoma, although they were reported as malignant. Geipel (1931), Boemke (1933), Roux-Berger and Debroise (1936), and Hollmann (1936) all gave examples of bronchial tumours which probably belonged to this group. In 1917 Jackson described a so-called 'endothelioma' of the bronchus, but in a recent paper (Jackson and Konzelmann, 1937) he stated that he now regarded this tumour as an adenoma. Patterson, reviewing the literature in 1930, found only three adenomata among 26 benign bronchial tumours, a figure which again suggests that many must have been mistaken for carcinoma at this time. On the other hand, there are examples of true carcinomata with survival for a few years, where

the diagnosis cannot be questioned (Ormerod, 1937). Confusion is likely to occur, however, until it is generally recognized that bronchial adenomata show degrees of differentiation, and that a glandular structure is not the only criterion for identification. The prevalent type of cell, the benign architecture, and above all the clinical picture must be taken into account.

The appearance of a biopsy specimen taken from a bronchial adenoma may vary according to the part of the tumour from which it was removed. The majority of such specimens are taken from the periphery, where infection and pressure, together with artificial influences such as squeezing by forceps or diathermy coagulation, produce distortion, and where the structure is normally somewhat irregular. Often there are inflammatory changes and the degenerate nuclei of the tumour cells tend to be small, deeply stained, or pyknotic. As a result of distortion and tissue damage these atypical cells appear to infiltrate the stroma in a way that simulates malignancy. Edwards (1938) has remarked on the comparative ease with which these tumours are recognized under the microscope by an observer who has had the opportunity of examining a number of them. Their recognition depends not so much on a specialized structure, but on certain fundamental features which are common to all bronchial adenomata. These features are uniformity in the type and staining reactions of the cells, a tendency to develop along glandular lines, absence of nuclear irregularities and mitoses, and absence of invasion of the blood-vessels or of the lymphatic system, including the bronchial lymph nodes.

*Malignancy.* Adenoma of the bronchus is usually included among the benign bronchial tumours, but many writers have regarded this growth as potentially malignant, although the supporting evidence is unconvincing. Distant metastases have never been recorded, so that it is necessary to discuss only the possibility of local malignancy. The presence of small areas of nuclear hyperchromatism and occasional mitoses in a bronchial adenoma have been reported by Reisner (1928) and Gower (1937). Infiltration of the bronchial wall by these tumours has been described by Kramer (1930), Zamora and Schuster (1937), and Brock (1938), but in all these instances the infiltration was extremely localized. Wessler and Rabin (1932) reported two cases of bronchial carcinoma in which the disease was of several years' duration and produced no metastases; no detailed histological description was given, but the authors thought that these growths originated as bronchial adenomata. Womack and Graham (1938) described examples in which invasion of the blood-vessels and lymphatic permeation by the tumour cells were observed. Since the authors made no distinction between adenoma and certain forms of carcinoma the value of these records is questionable. Kramer and Som (1935) in their report of 23 examples of bronchial adenoma made no mention of potential malignancy; Clerf and Crawford (1936) reported one instance in which the growth was observed for many years and did not become malignant; and finally, Jackson and Konzelmann (1937), writing from a wide experience of bronchial tumours, stated that they had

never seen any evidence of infiltration or metastasis occurring with a bronchial adenoma.

Only two tumours in the Brompton Hospital series showed any features which could be construed as malignant (Nos. 1 and 14) and these have already been cited in a recent paper by Brock (1938). In the first patient small submucous plaques of adenoma tissue were seen in the bronchus above the actual growth, which tended to recur after removal. These plaques must have arisen by a process of infiltration and therefore the growth was malignant in the strict sense of the term. It is significant that the duration of the disease prior to diagnosis in this patient was certainly two years and probably four years, and that the man is still alive and well more than four years later. In the second instance similar submucous extension was described, but haemorrhage and infection made it difficult to obtain a clear view of the tumour. The patient died of pneumonia after a haemoptysis and no infiltration was found at necropsy; here again the adenoma had produced symptoms for six years. No others showed any definite infiltration and although the tumours were generally very vascular and numerous thin-walled blood-vessels were present in close association with the tumour cells, the latter were never observed within the lumen of a vessel, nor were the lymphatics invaded. Therefore it may be said that bronchial adenomata may reasonably be regarded as benign tumours, since they have a long life-history and have never been known to cause death by direct or metastatic extension. Slow infiltration of the bronchial wall may occur, but it plays no part in the course of the disease.

*Origin.* Two theories have been advanced to account for the origin of bronchial adenomata. The earlier workers regarded them as tumours of the bronchial mucous glands, but more recently it has been suggested that they have a developmental basis. Some resemblance to glandular structure is found in most of these tumours and first led to their being named adenomata. They do not appear to originate in the bronchial epithelium, since this is always present as a continuous layer over the surface of the growth, from which it is separated by an intact basement membrane. In bronchial carcinoma, transition between the epithelium and the tumour can often be demonstrated (Ormerod, 1937). Reisner (1928) and Kramer (1930) both considered that the most probable site of origin of bronchial adenomata was the secretory ducts of the bronchial glands, since the tumour cells resembled those of the ducts more closely than those of the alveoli. This was also true of the tumours forming the present series, but on the other hand, vacuolation of the cytoplasm was occasionally seen and in one example (No. 11) there were alveoli lined with high columnar epithelium and containing mucus. The presence of mucus has often been reported and was also seen in another case in the Brompton Hospital series (No. 16). Thus it would seem that bronchial adenomata are probably tumours of the whole gland, not merely of its ducts.

Churchill (1937) suggested that, since the microscopical appearance of these tumours bore a superficial resemblance to that of foetal lung, they

might be vestigial lobes occurring in the bronchial wall. No further evidence has been advanced to support this theory, and no tumour in the Brompton Hospital series contained any tissue suggestive of foetal lung. Womack and Graham (1938) thought that bronchial adenomata were similar to mixed tumours of the salivary glands and stated that they were therefore malignant and had a developmental origin, and also that they contained both entodermal and mesodermal structures. All the examples in the Brompton Hospital series appeared to be purely epithelial tumours; no mesodermal structures were found in them nor has such a finding been reported by any other worker. Nevertheless, the resemblance between adenomata of the bronchus and the so-called 'mixed' salivary tumours is striking, for both tumours have a tendency to glandular formation of the duct, to acinous type, and both can exist in varying degrees of differentiation. This close similarity has also been mentioned by Brock (1938) who described a tracheal growth which had the histological features of a mixed salivary tumour, and by Thomas (1940). Recent research on mixed tumours of the salivary glands suggests that they should be regarded not as malignant growths of developmental origin, but as benign adenomata. Patey (1930) showed that they contained only epithelial elements and had a marked tendency to form glandular structure. In his series of 55 examples he did not find one which showed any malignant tendency other than slight local infiltration. This view has been supported by Harvey, Dawson, and Innes (1938) who described these tumours as true adenomata of the salivary glands. They observed that the cells were undifferentiated, but might develop some degree of differentiation along glandular or epidermoid lines, or both. In spite of this variegated appearance, clinical malignancy was extremely rare, in the sense of metastases to the lymph nodes or distant parts. Recurrence was common, probably owing to the difficulty of removing all the growth.

This description of benign salivary tumours can be applied just as readily to bronchial adenomata, and the histological appearances are closely similar (Plate 12, Figs. 11 and 12). The glands of the trachea and large bronchi are comparable to the salivary glands developmentally. They are composed of both serous and mucous elements, in varying proportions, and even contain crescents of Gianuzzi. Therefore, it seems reasonable to conclude that bronchial adenomata and benign salivary tumours are identical in nature. This hypothesis also links together the numerous pleomorphic epithelial tumours of the bronchus which have been described under so many different titles in the past. Some of these cannot be called adenomata, since they have no glandular structure, but they are benign epithelial tumours and often closely resemble the less differentiated adenomata. Pleomorphism and lack of structural differentiation are common in benign tumours of the salivary glands, and it is therefore to be expected that they will also be seen in tumours of the tracheal and bronchial glands. The structure of each individual bronchial gland tumour tends to be uniform: thus one growth may be composed of undifferentiated elements only, while another may be entirely

glandular, but it is uncommon to find both types of structure in the same tumour. Here they resemble the unmixed rather than the mixed type of salivary tumour, and Thomas (1940) has remarked upon this similarity, pointing out that both bronchial adenomata and unmixed salivary tumours are very radio-sensitive, whereas the mixed salivary tumour is resistant to irradiation.

### *Treatment and Results*

The treatment of an uncomplicated bronchial adenoma should have two objects, the removal of the obstructing tumour and the prevention of recur-

TABLE V

#### *Results of Treatment*

	Recorded cases		Brompton Hospital series		Total	
	Number	Result	Number	Result	Number	Result
<i>Simple removal</i>	12		3		15	
Cured		8		1		9
Improved		3		0		3
Died		1		2		3
<i>Removal and diathermy</i>	10		0		10	
Cured		7		0		7
Improved		3		0		3
<i>Removal and radon or radium</i>	9		13		22	
Cured		7		2		9
Improved		2		8		10
Died		0		3		3
<i>Removal and deep X-ray treatment</i>	7		2		9	
Cured		5		2		7
Improved		1		0		1
Stationary		1		0		1
<i>Removal, radon, and X-ray treatment</i>	2		2		4	
Cured		1		0		1
Improved		1		1		2
Died		0		1		1

rence. Good results after simple bronchoscopic removal have been reported by many authors (Table V), but such treatment is often followed by recurrence. This is probably due to two factors; in the first place small portions of the growth may easily be left embedded in the mucosa, since the tumour is extracted piecemeal with forceps resulting in profuse bleeding which obscures the view and makes complete eradication of the tumour extremely difficult. Also, the growth may have an unsuspected extrabronchial extension which cannot be removed through the bronchoscope. An unusual example of a cure by simple removal occurred in the Brompton Hospital series (No. 11). The growth was thought to be a localized carcinoma and was exposed by opening the chest and incising the lung. A firm, pedunculated mass was found arising within a dilated bronchus; this was excised, the lung was

sutured, and the chest closed. The patient made a rapid recovery and is alive and well 10 years later.

Excision by surgical diathermy, applied through the bronchoscope, has been advocated, since by this means bleeding is controlled and the cauterization of the bronchial wall tends to prevent recurrence. Good results have been reported, but the procedure is dangerous because the manipulation of a cautery within the lumen of a bronchus is a difficult undertaking and, when removing tumour tissue from the bronchial wall, it is easy to perforate one of the large blood-vessels. An example of this accident occurred in the Brompton Hospital series (No. 2). Repeated diathermy renders the tissues atrophic and may cause the rupture of a large vessel into a bronchus.

Local irradiation by means of radium or radon seeds has been used extensively in the treatment of bronchial adenoma. Radon is the more satisfactory and seeds can be inserted either in a tubular container or directly into the growth. Irradiation of the tumour causes shrinkage and atrophy, and facilitates subsequent removal by forceps, while irradiation of the bronchial wall tends to prevent recurrence. The best method of application is by means of a container, since this can be placed exactly in the desired position and the dosage can be regulated by the amount of radon inserted and the length of application. The disadvantages are that the container is sometimes expectorated and that its introduction may be difficult when the tumour is occluding the bronchus.

Deep X-ray therapy alone does not appear to have been tried, but as an adjunct to bronchoscopic removal, instead of the local application of radon, it has had a few successes. However, as Brock (1938) has pointed out, it is hardly justifiable to irradiate a large area of the chest for so small a tumour when it is possible to attack it locally.

Up to the present time a radical operation has rarely been employed in the treatment of bronchial adenoma. Kernan (1935) reported three instances in which such a procedure was carried out, in two of which the patients died, while the third was cured. Five of the Brompton Hospital patients were so treated. In one instance (No. 5) the tumour and the bronchiectatic lobe distal to it were both removed and the patient made a good recovery. In another (No. 7), a successful lobectomy was performed for residual bronchiectasis five years after the tumour had been removed through the bronchoscope. In the remaining three patients the operation had to be abandoned owing to dense pleural adhesions; in two of these (Nos. 12 and 17) the adhesions were due to a previous artificial pneumothorax, while the third patient (No. 21) had a gross suppurative bronchiectasis of many years' duration and the operation proved fatal.

Table V summarizes and compares the results of various forms of treatment. The average period of observation after treatment was three to four years; Jackson and Konzelmann (1937) described a patient who was alive and well 12 years after diagnosis, while the longest cure in the Brompton Hospital series was 10 years (No. 11). Of the 22 Brompton Hospital patients,

all of whom have been followed to date, 15 are alive and seven are dead. Four had fatal haemoptyses, one of which was due to the rupture of an artery after the application of diathermy (No. 2), two resulted from attempts to dilate bronchial strictures caused by radon (Nos. 6 and 10), and the fourth followed drainage of a chronic pulmonary abscess two years after diagnosis (No. 8). A fifth patient who died (No. 14) also had a large haemoptysis after drainage of a lung abscess, shortly after which he developed a fatal pneumonia. Another patient (No. 13) died of carcinoma of the stomach six years after the adenoma had been removed, during which time she had been free from symptoms. The last death in this series (No. 21) was due to shock after attempted lobectomy. The other 15 Brompton Hospital patients are still alive and able to follow their normal occupations; five are completely free from symptoms and the rest are improved. The duration of the disease in these patients varied from one to 25 years, with an average of nine years; the average duration since diagnosis is  $4\frac{1}{2}$  years.

The results given above suggest that a bronchial adenoma should be treated as follows. Removal by bronchoscopic forceps should be attempted first, unless there are complications. If it is possible, a radon container should then be inserted into the bronchus to irradiate the site of the growth. Brock (1938) advised a dose of  $12 \times 1$  millicurie seeds with a total screenage of 0.6 mm. of platinum, left in position for five to seven days. When bleeding is too free to allow removal by forceps, radon should be inserted in order to shrink the tumour and render it less vascular, and if the growth is large enough to prevent the insertion of a container, radon seeds may be thrust into it by means of a bronchoscopic gun. When this irradiation has had its effect, the adenoma may be removed piecemeal, and the bronchial wall irradiated once more with a container. The patient should be bronchoscoped again three months later and, if the tumour has recurred, the treatment should be repeated. This may be done three or four times, but not more because of the danger of overdosage. At least two applications of radon are usually necessary, unless the tumour is very small; eight of the 13 Brompton Hospital patients who were treated with radon required more than one application. If radon is applied too often it causes a stricture of the bronchus, and this occurred in two of the Brompton Hospital patients (Nos. 6 and 10). In both instances an attempt to dilate the stricture with bougies caused the rupture of a large vessel and the patients died. Both these patients were first treated nine years ago, at a time when gold-coated radon seeds were being used in a German-silver container. The incidence of radio-necrosis has now been much reduced by the use of platinum screens both in the seeds and applicator.

Continued recurrence of an adenoma, in spite of radon treatment, probably indicates the presence of an extrabronchial extension of the growth. Even if the portion of the tumour outside the bronchus is only a few millimetres in diameter, it will not be accessible through the bronchoscope and local irradiation may not be sufficient to destroy it. In such circumstances,

repeated intrabronchial manipulation and its attendant risks will be necessary to prevent bronchial obstruction and secondary infection, and the most effective treatment is to remove that portion of the lung which contains the growth.

It has been argued that the tendency of some bronchial adenomata to become locally malignant demands that lobectomy or even pneumonectomy should be performed as soon as the tumour is diagnosed, but this would expose the patient with a small adenoma to unnecessary risk. No harm can result from attempting to remove the growth through the bronchoscope and then applying radon, for even if local infiltration has occurred the tumour will not produce metastases. The growth infiltrates so slowly and the process is so localized that moderate delay in its removal is of no moment, provided that bronchial obstruction can be prevented. Bronchoscopy at regular intervals will accomplish this object, and a certain proportion of cures will be obtained by simple removal combined with radon application. If the tumour does not respond to this treatment, a lobectomy can still be performed without increased risk. The possibility of radical surgical removal should always be borne in mind when considering the treatment of a bronchial adenoma. Modern technique has so greatly reduced the death-rate from lobectomy that Edwards (1939) was able to report a mortality of only 15 per cent. in a series of lobectomies for infected bronchiectasis in adults. Much lower figures are obtainable when the operation is performed in the absence of infection. Pneumonectomy carries a higher mortality, but a lobectomy will often suffice to remove an adenoma, since the growth is confined to one bronchus and there are no glandular metastases. Surgical removal is the only satisfactory form of treatment for those bronchial adenomata which are sufficiently large to be visible radiologically; it is indicated when a tumour recurs repeatedly in spite of local irradiation, and also for complications such as bronchiectasis and chronic pulmonary abscess.

#### *Summary and Conclusions*

1. Bronchial adenomata form a distinct clinical group of tumours accounting for about five per cent. of all bronchial neoplasms detected by bronchoscopy, and are the commonest benign tumours of the bronchus. Twenty-two examples of this condition are described which have been seen at the Brompton Hospital in a series of 453 proved cases of bronchial neoplasm, an incidence of 4.8 per cent. These examples have been analysed and compared, together with those described in the literature.

2. Bronchial adenomata are benign tumours of the bronchial glands, and evidence is produced to suggest that they are probably identical in nature with salivary gland tumours. They all show certain histological characteristics, the chief being uniformity of structure and staining properties, a tendency to glandular formation, and absence of unruly growth. A limited infiltration of the bronchial wall has been observed, but metastatic spread is unknown and extension of the tumour is never the cause of death.



3. Only about a third of all bronchial adenomata have the highly differentiated glandular structure which has previously been regarded as typical of these tumours. The remainder are more or less undifferentiated and have in the past been regarded as bronchial carcinomata. Irrespective of these histological differences, all bronchial adenomata pursue the same benign clinical course. A suggested classification according to structure is described.

4. The symptoms and signs due to these growths tend to be protracted. They may be conveniently divided into two categories. The primary features are those due directly to the tumour, of which recurrent haemoptysis is the most important. The secondary features comprise two distinct groups, those due to bronchial obstruction with resulting pulmonary atelectasis, and those due to subsequent infection of the lung distal to the occlusion.

5. The prognosis is good only when treatment is instituted early. Delay results inevitably in the occurrence of bronchial obstruction, pulmonary suppuration, and eventual death. Recognition of these growths by bronchoscopy must be early if it is to forestall this sequence of events.

6. Evidence is produced that an uncomplicated adenoma of the bronchus is best treated by simple bronchoscopic removal combined with local irradiation. Lobectomy is the treatment of choice for those patients with tumours which recur after bronchoscopic removal, for those with large extrabronchial tumours, and for those with a lung already damaged by secondary infection.

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## APPENDIX I

*Summary of Cases of Bronchial Adenoma Collected from Published Records*

NOTE.—The following abbreviations have been used in the Appendices :

R. = right      L. = left      R.U.L. = right upper lobe      R.M.L. = right middle lobe  
 R.L.L. = right lower lobe      L.U.L. = left upper lobe      L.L.L. = left lower lobe

No.	Author and date	Sex	Age	Duration before diagnosis	Haemoptysis	Other symptoms
1	Mueller, 1882	F.	22	8 years	+	Cough
2	Jackson, 1917	M.	35	5 years	+ recurrent	Wheezing
3	Heine, 1927	M.	44	?	0	None
4	Reisner, 1928	F.	47	5 years	+	Cough and pleurisy
5	Kramer, 1930	F.	36	13 years	+ recurrent menstrual	Pleurisy with effusion, pyrexial attacks
6	Ditto	M.	26	5 years	+	Pneumonia, dyspnoea, cough, pleurisy
7	Wessler and Rabin, 1932	F.	36	13 years	+ recurrent menstrual	Pleurisy with effusion, pyrexial attacks
8	Ditto	F.	17	5 years	+ recurrent menstrual	Cough, sputum, and fever
9	Ditto	M.	26	3 years	+	Pleurisy with effusion, pneu- monia, dry cough
10	Ditto	F.	47	5 years	+	Pleurisy, empyema, amyloid disease
11	Ditto	F.	27	11 years	+	Pleurisy and pneumonia, cough and sputum, dyspnoea
12	Ditto	F.	44	6 months	0	Cough and sputum, clubbing of fingers
13	Ditto	M.	48	2 weeks	0	Cough, fever, and pain in chest
14	Ditto	M.	35	?	?	Cough and profuse purulent sputum
15	Ditto	M.	32	7 years	+	Dry cough and fever
16	Ditto	F.	35	2 years	+	Cough, pain, fever, and wheez- ing
17	Fried, 1934	M.	57	Several months	?	Cough and dyspnoea
18	Kernan, 1935	F.	35	4 years	0	Attacks of acute dyspnoea
19	Ditto	F.	17	6 years	+ recurrent	None
20	Ditto	F.	54	40 years	+ recurrent	None
21	Ditto	F.	33	1 year	+	Cough and offensive sputum
22	Ditto	F.	?	?	0	Wasting and anorexia
23	Ditto	F.	58	2 weeks	0	Cough and dyspnoea
24	Ditto	M.	?	2 weeks	+	Acute febrile onset
25	Ditto	M.	?	1 year	0	Pneumonia, cough, and py- rexial attacks
26	Ditto	F.	26	1 year	0	Cough, dyspnoea, and pyrexial attacks

Pulmonary lesion	Bronchus affected	Histo-logical group	Treatment	Duration since diagnosis	Result	No.
Bronchiectasis	L. main	1	—	—	—	1
Recurrent collapse of R.L.L.	R. main	2	Bronchoscopic removal	?	Recovery	2
Collapse of R.M.L., bronchiectasis	R.M.L.	1	—	—	—	3
Bronchiectasis R.L.L., empyema	R.L.L.	1	Drainage of empyema	—	Died, haemorrhage	4
Consolidation of R.M.L.	R.L.L.	?	Removal and radon	1½ years	Cured	5
Massive collapse L. lung	L. main	?	Radium alone	?	Cured	6
Collapsed R.M.L.	R.M.L.	?	Removal	?	Cured	7
Collapsed R.L.L.	R.L.L.	?	None, removal failed	?	Stationary	8
Collapsed L.L.L.	L.L.L.	?	Removal and radium	6 years	Cured	9
Collapsed L.L.L., bronchiectasis	L.L.L.	?	Drained as lung abscess	—	Died, haemorrhage	10
Massive collapse L. lung	L. main	?	Refused treatment	?	Stationary	11
Collapsed R.L.L.	R.L.L.	?	—	—	—	12
L. pyo-pneumothorax	L.L.L.	?	Drainage of pyo-pneumothorax	—	Died	13
R. broncho-pneumonia and abscess	R.L.L.	?	Drainage of lung abscess	—	Died	14
Collapsed R.L.L.	R. main	?	Deep X-ray, radium to recurrence	7 years	Improved	15
Collapsed R.L.L., bronchiectasis	R.L.L.	?	—	—	—	16
Bronchitis and emphysema	L. main	1	None	—	Died, heart failure	17
Recurrent collapse L. lung	L. main	?	Diathermy and radon	4 years	Cured	18
Collapsed R.L.L.	R.L.L.	?	Deep X-ray	4 years	Cured	19
Collapsed R. lung, bronchiectasis	R. main	2	Pneumonectomy	—	Died	20
Bronchiectasis of L.L.L.	L.L.L.	?	Lobectomy	6 years	Cured	21
Collapsed R.L.L.	R. main	?	Diathermy and radon (bronchial stricture)	4 years	Improved	22
Massive collapse L. lung	L. main	2	Removal	4 years	Cured	23
Recurrent collapse of L.L.L.	L. main	2	Diathermy, lobectomy	4 years	Died	24
Pneumonia L.U.L.	L.U.L.	2	Removal and diathermy	1½ years	Cured	25
Intermittent collapse R. Lung	R. main	?	Diathermy	3 months	Cured	26

APPENDIX I (continued)

No.	Author and date	Sex	Age	Duration before diagnosis	Haemoptysis	Other symptoms
27	Rosenblum and Klein, 1935	M.	11	10 months	+	Cough, sputum, fever, and wasting
28	Gerlings, 1936	F.	39	3 years	+ recurrent menstrual	Pleural pain
29	Gowar, 1937	F.	37	2 years	+	Cough and pleurisy
30	Ditto	F.	23	2 months	0	Pleurisy and pneumonia, cough and sputum
31	Ditto	M.	24	7 years	+	Cough
32	Zamora and Schuster, 1937	F.	30	2 years	+	Cough, pain, dyspnoea, and wheezing
33	Ditto	F.	54	4 years	+	Recurrent broncho-pneumonia, 'asthma'
34	Jackson and Konzelmann, 1937	F.	24	4 years	+ large	Cough and pain
35	Ditto	F.	38	3 years	+ recurrent menstrual	Pneumonia, wasting, pyrexial attacks, cough
36	Ditto	F.	15	5 months	0	Cough and sputum, wasting, pyrexial attacks
37	Ditto	F.	25	2 years	+	Cough
38	Ditto	F.	32	2 years	+ recurrent menstrual	'Influenza' and pleurisy
39	Ditto	F.	38	2½ years	+	Cough, pain, and dyspnoea
40	Ditto	F.	45	5 years	+	Cough and sputum, pain and wasting
41	Ditto	F.	56	18 months	+	Cough and sputum
42	Ditto	F.	28	2 years	+	Cough and sputum, pain and pyrexia
43	Ditto	F.	33	2 years	0	Cough, sputum, and pyrexia
44	Ditto	F.	42	1 year	0	Wasting, dry cough, wheezing
45	Hart, 1937	F.	47	4 months	0	Cough and pyrexial attacks
46	Mallory, 1938	M.	23	8 years	+ recurrent	Pneumonia, dyspnoea, wheezing, cough, and sputum
47	Tchertkoff and Klosk, 1939	F.	48	10 years	+ recurrent profuse	Pneumonia and empyema

The following examples have also been reported, but without giving details of each individual patient:

Kramer and Som (1935): 23 examples. Sex, nine male and 14 female. Age, 13 to 64 years, the majority occurred in the third and fourth decades. Duration before diagnosis, five months to 30 years. Haemoptysis, present in 19 patients. Other symptoms included cough, offensive sputum, asthma, and recurrent pneumonia. Pulmonary lesion, atelectasis (13), pneumonia (3), empyema (2), pneumonitis (2), bronchiectasis (2), negative (1). Site of growth, bronchus of first or second order. Treatment included

Pulmonary lesion	Bronchus affected	Histo-logical group	Treatment	Duration since diagnosis	Result	No.
Collapsed R.L.L., bronchiectasis	R. main	1	Expelled after bronchoscopy	2 months	Improved	27
Collapsed L.L.L.	L. main	1	Removal	2 months	Cured	28
Collapsed R.L.L.	R.L.L.	2	Removal and radon	2 years	Cured	29
Collapsed R.L.L. and R.M.L.	R.L.L.	2	Removal	6 months	Cured	30
Collapsed R.L.L.	R.L.L.	1	Removal and radon	?	—	31
Massive collapse L. lung	L. main	2	Removal and radon	2 months	Improved	32
Collapsed R.U.L.	R. main	?	Removal	—	Died, pneumonia	33
Partial collapse R.L.L.	R. main	2	Removal and deep X-ray	12 years	Cured	34
Pleural effusion at L. base	L. main	2	Removal and deep X-ray	8 years	Cured	35
Collapsed R.L.L.	R. main	2	Removal	6 years	Cured	36
Collapsed R.L.L.	R.L.L.	2	Removal and diathermy	5 years	Improved	37
Collapsed L.L.L.	L.L.L.	1	Removal	4 years	Cured	38
Obstructive emphysema L. lung	L. main	2	Removal failed, deep X-ray	2½ years	Improved	39
Collapsed L.L.L., bronchiectasis	L. main	1	Removal, radon, and deep X-ray	1 year	Cured	40
Emphysema, collapse, and effusion	R. main	2	Removal and deep X-ray	2 years	Stationary	41
Collapsed R.L.L.	R. main	2	Removal	—	—	42
Collapsed L.U.L.	L.U.L.	2	Removal	6 months	Improved	43
Radiological opacity L. lung	L. main	1	Removal and deep X-ray	1 month	Cured	44
Collapsed R.U.L.	R.U.L.	2	Removal and silver nitrate	9 months	Cured	45
Massive collapse L. lung	L. main	?	Removal, diathermy, radon, and deep X-ray	?	?	46
Collapsed R.L.L.	R. main	1	Diathermy	?	Improved	47

removal, diathermy, radium, and deep X-ray. Duration since diagnosis averaged nine years. Results, 17 patients living and six dead (four died after thoracotomy, one from pneumonia, and one from haemoptysis).

Clerf and Crawford (1936): 17 examples. Sex, nine male and eight female. Age, 21 to 40 years. Duration and symptoms not noted. Pulmonary lesion, obstructive emphysema and atelectasis. Site of growth, five on right side, 12 on left. Lower lobe bronchus involved in nine. Treatment, removal. Results not noted.

## APPENDIX II

*Summary of Cases of Bronchial Adenoma seen at the Brompton Hospital*

No.	Sex	Age	Duration before diagnosis	Haemoptysis	Other symptoms
1	M.	45	2 years	+	Pneumonia, pyrexial attacks
2	M.	47	15 years	+ recurrent	Dyspnoea
3	F.	43	14 years	+	Pleurisy
4	F.	33	1 year	+	Pleurisy, cough, and sputum
5	M.	46	3 years	+	Pneumonia, cough and sputum, asthma, arthritis
6	M.	56	1 year	+	Pleurisy, pneumonia, cough, and dyspnoea
7	F.	34	10 years	+	Recurrent pleurisy, cough and sputum, dyspnoea
8	F.	51	6 months	+	Cough and sputum, pyrexia
9	M.	31	18 months	0	Dry cough and increasing dyspnoea
10	M.	57	5 years	+	Recurrent pleurisy, cough and sputum, dyspnoea
11	F.	27	6 months	+	Dry cough and dyspnoea
12	M.	35	18 months	+	Cough and sputum
13	F.	66	2 months	+	Cough and sputum, dyspnoea
14	M.	32	6 years	+ large	Recurrent attacks of pyrexia
15	M.	49	9 years	+ recurrent	None
16	F.	50	8 months	0	Pyrexial attack, cough and sputum
17	M.	36	13 years	+ large	Pyrexial attack, cough and sputum
18	M.	33	2 years	+	Pleurisy, cough, and foul sputum
19	F.	52	23 years	+ recurrent menstrual	Cough, pleurisy, pyrexial attacks
20	F.	33	3 years	+	Pyrexial attacks, cough
21	M.	35	14 years	+	Pleurisy, pyrexial attacks, cough and sputum
22	F.	22	6 months	+ recurrent	Pneumonia, pleural effusion

Pulmonary lesion	Bronchus affected	Histo-logical group	Treatment	Duration since diagnosis	Result	No.
R. empyema, collapsed R. lung	R. main	2	Diathermy and deep X-ray	3 years	Cured	1
Collapsed R.L.L.	R. main	2	Diathermy, deep X-ray, and radon	3 months	Died, haemoptysis	2
Collapsed R.L.L.	R. main	1	Removal, radon, and deep X-ray	3 years	Improved	3
Partial collapse of R.L.L.	R.L.L.	2	Removal and radon	7 years	Cured	4
Collapsed R.L.L., bronchiectasis	R.L.L.	2	Removal, radon, and R.L. lobectomy	2 years	Improved	5
Collapsed R.L.L.	R.L.L.	1	Removal, radon (bronchial stricture)	3 years	Died, haemoptysis	6
Collapsed R.L.L., bronchiectasis	R.L.L.	2	Diathermy, 5 years later R.L. lobectomy	9 years	Cured	7
Radiological opacity in R.L.L.	R.L.L.	2	Drainage of lung abscess, radon	2 years	Died, haemoptysis	8
Collapsed R.U.L.	R. main	2	Removal and radon	5 years	Improved	9
Collapsed R.L.L.	R.L.L.	1	Radon (bronchial stricture)	5 years	Died, haemoptysis	10
Rounded tumour in L. upper zone	L.U.L.	1	Thoracotomy and removal	10 years	Cured	11
L. artificial pneumothorax	L.U.L.	2	Removal and deep X-ray	8 years	Cured	12
Collapsed R.M.L.	R.M.L.	2	Removal	6 years	Died, another disease	13
Collapsed R.L.L.	R.L.L.	2	Removal, drainage of lung abscess	6 months	Died, pneumonia	14
Partial collapse of R.L.L.	R.L.L.	2	Removal and radon	2 years	Improved	15
Collapsed R. lung	R. main	2	Removal and radon	2 years	Improved	16
Collapsed R.L.L., bronchiectasis	R. main	1	Removal and radon, lobectomy failed	8 years	Improved	17
Collapsed R.L.L.	R.L.L.	2	Removal and radon	3 years	Cured	18
Collapsed R.L.L.	R.L.L.	2	Removal and radon	1 year	Improved	19
Collapsed L.L.L.	L.L.L.	2	Removal and radon	9 months	Improved	20
Collapsed R.L.L. and R.M.L.	R.M.L.	2	Attempted R. pneumonectomy	—	Died, shock	21
Collapsed L.L.L., bronchiectasis	L.L.L.	1	Removal and radon	6 months	Improved	22

## APPENDIX III

*Case Protocols*

CASE 1. A man, aged 45 years, lorry driver.

*History.* In 1932 'pneumonia' on R. side, followed by a pleural effusion and later a persistent cough. In 1934 an acute febrile attack, followed by a small haemoptysis. Admitted to Brompton Hospital in November 1934.

*Physical examination.* General condition good. Dullness to percussion over R.L.L., heart and trachea displaced to R.

*X-ray.* R. hydropneumothorax.

Thick pneumococcal pus was withdrawn from R. pleural cavity, after which the empyema was drained surgically. One year later the cavity was no smaller.

*Bronchography* (March 1936). Cup-shaped occlusion of R. main bronchus.

*Bronchoscopy.* Submucous tumour, 1.5 cm. in diameter, arising from the lateral wall of the R. main bronchus 1 cm. below the carina. This was thought to be a carcinoma; it was removed by diathermy, and a course of deep X-ray therapy was given.

One year later there was a stenosis of the R. main bronchus surrounded by multiple small projections of growth, the highest at the level of the carina. These were removed and bronchoscopy in 1938 showed only a very small recurrence.

In January 1940 there was still considerable collapse and fibrosis of the R. lung, but the patient was well and had no symptoms.

*Histology.* The covering epithelium is squamous. There is an intermediate layer of loose, cellular fibrous tissue containing irregular masses of cells. The tumour proper consists of cell masses arranged in strands and columns with a tendency to form alveoli. There is a moderate amount of fibrous stroma. The tumour cells are mostly small and flattened or polyhedral, those of the alveoli are cuboidal with large rounded nuclei containing granular chromatin. At the periphery many of the cells are degenerate and pyknotic, but otherwise the structure is uniform and no mitoses or other signs of activity can be seen. This is an example of the intermediate type of adenoma (Group 2).

CASE 2. A man, aged 47 years, engineer.

*History.* In 1918 influenza followed by chronic productive cough. In 1919 'double pneumonia' followed by R. pleural effusion. In 1921 'pneumonia' on R. side with haemoptysis. During the next 15 years he had recurrent large haemoptyses, occasional pain in R. chest, and dyspnoea on exertion. Admitted to Brompton Hospital in September 1936.

*Physical examination.* General condition good. Dullness to percussion, diminished breath sounds, and some rales over R.L.L.

*X-ray.* Collapse of R.L.L.

*Bronchography.* Occlusion of R. descending bronchus.

*Bronchoscopy.* Smooth, vascular, polypoid mass in R. descending bronchus. Five 2 mc. radon seeds were inserted into growth and a course of deep X-ray therapy was given.

Three months later, haemoptysis of 2½ pints. An attempt was made to remove the tumour by diathermy, but the patient died from a fatal haemoptysis 24 hours after this operation.

*Autopsy* (Plate 9, Fig. 6). Smooth pedunculated mass the size of a pigeon's egg was found arising from the anterior wall of the R.L.L. bronchus and extruded upwards into the main bronchus. The bronchial wall was thin and there was a perforation in it, probably due to the separation of a slough, which opened into the pulmonary artery. The R.L.L. was collapsed and contained calcified tuberculous deposits. The regional lymph nodes showed no histological evidence of growth.

*Histology.* The epithelium is squamous in parts and beneath it there is a layer of very vascular connective tissue. The tumour proper consists of masses of cells in a scanty



fibrous stroma. Many large blood-vessels are present in close relation to the tumour cells, but there is no invasion of the vessel walls. The cells themselves are polyhedral with large clear nuclei containing finely granular chromatin. In some areas there are well-formed alveoli with basement membranes while in others the cells are arranged in twisted columns and solid masses. No mitoses can be seen and nuclear hyperchromatism is absent except in degenerate areas at the periphery. There is no infiltration of the bronchial wall. The appearances are those of a bronchial adenoma showing moderate differentiation (Group 2).

CASE 3. A married woman, aged 43 years.

*History.* In 1922 a sudden large haemoptysis. In 1923 pleurisy and pneumonia on R. side, followed by intermittent R. pleural pain for 4 years. In 1927 diagnosed as pulmonary tuberculosis and sent to sanatorium for three months. In 1930 large haemoptysis followed by recurrent attacks of pleurisy on R. side and repeated small haemoptyses. Admitted to Brompton Hospital in January 1936.

*Physical examination.* General condition good; slight clubbing of the fingers. Impaired percussion note and diminished air-entry over R.L.L.

*X-ray.* Collapse of R.L.L.

*Bronchography.* Complete occlusion of R.L.L. bronchus.

*Bronchoscopy.* Lobulated mass arising in R.L.L. bronchus and extending into the main and M.L. bronchi. Six 2 mc. radon seeds were inserted into the growth and this dose was repeated two months later, after which a course of deep X-ray therapy was given.

In May 1940 the patient still had occasional pain and stained sputum, but otherwise she was well. It was felt that the only possibility for further radical treatment would be a pneumonectomy, and this was contra-indicated by the patient's age.

*Histology.* The specimens from this patient are fragmentary. The remains of the covering epithelium are stratified. There is a very vascular intermediate layer of loose connective tissue containing islets of adenoma cells. The tumour proper is a highly differentiated adenoma (Group 1). The cells are arranged to form alveoli and tubules closely packed together in a fibrous stroma; they are mostly cuboidal with large round or oval nuclei containing granular chromatin. The cytoplasm is scanty and vacuolated in parts, but no mucus is present in the alveoli. Basement membranes are well marked throughout and there is no invasion of the blood-vessels. Mitoses are absent and the nuclei everywhere are even in size and shape.

CASE 4. A married woman, aged 33 years.

*History.* In 1931 influenza and pleurisy on R. side, followed by a chronic productive cough and repeated small haemoptyses. Admitted to Brompton Hospital in October 1935.

*Physical examination.* General condition good, fingers not clubbed. Impaired percussion note and weak breath sounds over R.L.L.

*X-ray.* Partial collapse of the R.L.L.

*Bronchography.* Partial obstruction of the R.L.L. bronchus.

*Bronchoscopy.* Partially pedunculated mass, almost occluding the R.L.L. bronchus. Four 1.5 mc. radon seeds were inserted into the growth and one month later the growth was removed.

In May 1940 the patient was well and free from symptoms, no growth could be seen on bronchoscopy, and an X-ray showed normal translucency at both bases.

*Histology.* The specimen consists of part of the tumour proper; the epithelium and outer layers are missing. It is composed of strands and masses of cells in a scanty fibrous stroma which contains some thin-walled blood-vessels. There are a few definite alveoli and many of the cells are arranged in rosettes as though attempting alveolar structure. The cells themselves are uniform in size and shape, they are mostly cuboidal with characteristic nuclei. No mitotic figures or signs of invasion are present, and the picture is one of an adenoma showing a moderate degree of differentiation (Group 2).

CASE 5. A man, aged 46 years, dental surgeon.

*History.* In 1934 gradual onset of productive cough, repeated small haemoptyses, and occasional attacks of dyspnoea of an asthmatic type. In 1935 he began to have recurrent pains in the joints. In 1936 'pneumonia' on the R. side, after which the sputum became offensive. Admitted to Brompton Hospital in April 1937.

*Physical examination.* General condition good, fingers not clubbed; slight dyspnoea on exertion. Some diminution of air entry over R.L.L. Evidence of arthritis in the finger joints and in the R. wrist, elbow, and knee.

*X-Ray.* Patchy collapse and fibrosis in R.L.L.

*Bronchography.* Complete occlusion of R.L.L. bronchus.

*Bronchoscopy.* Vascular polypoid tumour in R.L.L. bronchus: this was removed piecemeal. The arthritis improved immediately and in July 1937 twelve 1.5 mc. radon seeds were inserted in a container for 26 hours. The tumour recurred repeatedly in spite of radon treatment and in June 1938 a R.L.L. lobectomy was performed. At operation a small piece of growth was found outside the bronchus and was removed. Subsequent bronchoscopy showed no sign of growth, and in February 1940 the patient was still alive and his condition improved.

*Histology.* The covering epithelium is partly columnar and partly squamous; beneath this there is an intermediate layer of fibrous tissue containing blood-vessels and numbers of tumour cells arranged in irregular strands. The tumour proper is very cellular, the cells are mostly cuboidal with scanty cytoplasm and clear oval nuclei containing granular chromatin. Mitoses are absent and the nuclei are even in size. The structure consists of alveoli and solid acini in a scanty vascular stroma. At the periphery the arrangement of cells is much more irregular; some are degenerate and have pyknotic nuclei and the appearance might be mistaken for malignancy. The general structure is that of a moderately differentiated adenoma (Group 2).

CASE 6. A man, aged 56 years, butcher.

*History.* In January 1930 pleurisy and broncho-pneumonia on the R. side, followed by an empyema. This was drained and healed well, but he was left with a chronic cough and, in March 1931, commenced to have small haemoptyses with increasing dyspnoea. Admitted to Brompton Hospital in July 1931.

*Physical examination.* General condition good. Dullness to percussion, weak breath sounds, and some fine rales over R.L.L.

*X-ray.* Collapse of R.L.L.

*Bronchography.* Occlusion of R.L.L. bronchus.

*Bronchoscopy.* Smooth mass growing from wall of R.L.L. bronchus. This was removed and twelve 1.5 mc. radon seeds were inserted in a container for eight days.

One year later, bronchoscopy showed a stricture of the bronchus and a small recurrence of the tumour; twelve 1.5 mc. radon seeds were applied as before and this dose was repeated.

In October 1934 the patient had a pyrexial attack due to retention of secretion beyond the stenotic bronchus; an attempt to dilate the stricture resulted in a fatal haemoptysis.

*Autopsy.* The R.L.L. was collapsed and bronchiectatic. There was radionecrosis at the site of the bronchial stricture, but no sign of any growth could be found.

*Histology.* The fragment is a small part of the tumour proper. It consists of strands and masses of cells arranged in columns and whorls resembling tubules in a stroma of fibrous tissue. The cells are uniform in appearance and mostly cuboidal except in one part where there are definite alveoli lined by columnar cells. The nuclei are typical of a bronchial adenoma and show no mitotic figures or hyperchromatism and the fragment is probably derived from a tumour of the highly differentiated type (Group 1).

CASE 7. A married woman, aged 34 years.

*History.* In 1921 bronchitis and pleurisy on the R. side. During the next 10 years she gradually developed a productive cough and had six attacks of pleurisy on the R. side.

In January 1931 she developed persistent R. pleural pain, increasing dyspnoea, and loss of weight. Admitted to Brompton Hospital in March 1931.

*Physical examination.* General condition good, slight clubbing of the fingers. Dullness to percussion, diminished air entry, and harsh pleural friction over R.L.L.

*X-ray.* Partial collapse of R.L.L.

*Bronchography.* Filling defect in R.L.L. bronchus with distal bronchiectasis.

*Bronchoscopy.* Round, smooth, pink, vascular tumour attached to wall of R.L.L. bronchus; this was treated with wire diathermy. One month later a container with twelve 2 mc. radon seeds was inserted (length of application not noted).

Five years later, in 1936, the patient still had a cough with sputum and occasional attacks of pleurisy. No growth could be seen on bronchoscopy, but there was well marked bronchiectasis, for which a R.L.L. lobectomy was performed. In December 1939 the patient was well and free from symptoms.

*Histology.* The covering epithelium is thick and stratified; beneath it there is a dense layer of vascular fibrous tissue containing islets of degenerate tumour cells. The tumour proper consists of a mass of closely packed cells with very little stroma. The structure is uniform and there is some attempt at alveolar formation; rudimentary tubules are also present. The cells are mostly cuboidal and have characteristic nuclei, mitoses are absent and the blood-vessels are not invaded. The general structure is that of a moderately differentiated bronchial adenoma (Group 2).

CASE 8. A married woman, aged 51 years.

*History.* Productive cough for six months, with pyrexia and one small haemoptysis. Admitted to Brompton Hospital in April 1931.

*Physical examination.* General condition good, fingers not clubbed. Dullness to percussion and diminished air entry over R.L.L.

*X-ray.* Dense opacity in R. lower zone, heart and trachea displaced to the R.

*Bronchography.* Occlusion of the R.L.L. bronchus.

*Bronchoscopy.* Mass in the R.L.L. bronchus, thought to be granulation tissue.

An abscess in the R.L.L. was drained externally, but did not heal and the haemoptysis continued. Bronchoscopy two months later showed a nodular growth in the R.L.L. bronchus and a container holding twelve 1.5 mc. radon seeds was inserted for 10 days. The patient's condition did not improve, and in May 1936 she suddenly had a fatal haemorrhage from the chest sinus.

*Autopsy.* There was a white, rounded, encapsulated growth about the size of a walnut arising in the wall of the R.L.L. bronchus and extending both into the bronchus and into the lung. There was no invasion of lung tissue, and sections of the regional lymph nodes were normal. The R.L.L. was collapsed and bronchiectatic.

*Histology.* The tumour consists of isolated masses of cells in a delicate connective tissue stroma. The whole arrangement is very regular and the cells are typical of a bronchial adenoma; for the most part they are polyhedral with clear, scanty cytoplasm and large round or oval nuclei which contain a well-marked chromatin reticulum. In some of the cell masses there is a slight tendency to alveolar grouping. No mitoses or other signs of active growth are present. In view of the uniformity of structure and benign character of the cells, coupled with a slight tendency to glandular structure, this tumour is classed as an undifferentiated adenoma (Group 2).

CASE 9. A man, aged 31 years, clerk.

*History.* Dry cough and increasing dyspnoea for 18 months, but no haemoptysis. Admitted to Brompton Hospital in May 1934.

*Physical examination.* General condition good, fingers not clubbed. Impaired percussion note and weak bronchial breathing over R.U.L.

*X-ray.* Collapse of R.U.L.

*Bronchoscopy.* Pedunculated growth arising from orifice of R.U.L. bronchus; this was removed piecemeal. The next day there was a massive collapse of the R. lung which re-expanded after blood clot had been removed from the bronchus. Five 1.5 mc. radon

seeds were inserted into the bronchial wall and a further five 2 mc. seeds were introduced one month later.

In February 1940 the patient was well and free from symptoms although the R.U.L. was still partially collapsed.

*Histology.* The covering epithelium is transitional. The tumour proper is enclosed in a layer of vascular fibrous tissue in which there are irregular columns of tumour cells, arranged in rough alveoli. The tumour proper is composed of masses and columns of cuboidal cells with some well-formed alveoli, the cells of which are columnar. Nuclei are everywhere even in size and show no mitoses. This specimen is typical of a moderately differentiated bronchial adenoma (Group 2).

CASE 10. A man, aged 57 years, carpenter.

*History.* Chronic cough for many years. In 1927 and 1930 pleurisy on R. side. In 1931 he became dyspnoeic on exertion and had a small haemoptysis. Admitted to Brompton Hospital in May 1931.

*Physical examination.* General condition good. Dullness to percussion and diminished air entry over R.L.L.

*X-ray.* Collapse of R.L.L.

*Bronchography.* Occlusion of R.L.L. bronchus.

*Bronchoscopy.* Raspberry-like mass arising from wall of R.L.L. bronchus; this was partly removed and a container holding twelve 1.5 mc. radon seeds was inserted for seven days.

After this, the R.L.L. re-expanded and the patient remained well for two years, except for small haemoptyses.

In 1933 the symptoms recurred; bronchoscopy showed a recurrence of the growth and the radon treatment was repeated. The tumour did not recur again, but a bronchial stricture was formed and an attempt to dilate this, two years later, resulted in a fatal haemoptysis (March 1935).

*Autopsy.* There was no sign of any growth, but the fibrous stricture of the R.L.L. bronchus had so thinned the bronchial wall that a branch of the pulmonary artery had formed a small aneurysm projecting into the bronchus. This had perforated. The R.L.L. was collapsed and bronchiectatic.

*Histology.* The covering epithelium is squamous in parts; beneath it there is a narrow layer of vascular fibrous tissue which is infiltrated with inflammatory cells. The tumour proper consists of closely packed masses of cells in a fibrous stroma. The structure is glandular with well-formed alveoli and tubules. The cells are mostly large and polyhedral, their nuclei are typical and show no signs of activity. The whole appearance of the tumour is uniformly glandular and it is therefore classed as a highly differentiated bronchial adenoma (Group 1).

CASE 11. A married woman, aged 27 years.

*History.* In 1925 and 1926 'congestion of the lungs'. In 1929 she developed a dry cough, became dyspnoeic on exertion, and had a small haemoptysis. Admitted to Brompton Hospital in November 1929.

*Physical examination.* General condition good, fingers not clubbed. No abnormal physical signs in the chest.

*X-ray.* Clearly defined, rounded opacity in L.U.L.

A diagnostic L. artificial pneumothorax showed that the mass was intrapulmonary, and in January 1930 the L. chest was opened. A firm round pedunculated mass was found in the L.U.L. bronchus; it was excised, the lung was sutured, and the chest closed. The patient made an uninterrupted recovery.

In May 1939 the patient was alive and very well, apart from slight residual bronchiectasis in the L.U.L., which gave rise to occasional small haemoptyses.

*Histology.* The outer layers have not been preserved. The tumour consists of a mass of cells broken up by some fibrous trabeculae; it extends into the bronchial wall between the cartilages, but shows no sign of invasion. The cells are arranged in orderly columns

and strands, forming large numbers of tubules and alveoli. There are well-marked basement membranes and the cells are mostly columnar with typical nuclei; no mitotic figures can be seen. This is a striking example of a bronchial adenoma showing a very high degree of differentiation (Group 1).

CASE 12. A man, aged 35 years, civil servant.

*History.* In 1934 he developed a productive cough and vague constitutional symptoms; diagnosed as pulmonary tuberculosis and sent to a sanatorium where a L. artificial pneumothorax was induced. The cough persisted and the sputum became blood-stained occasionally. He also developed dyspnoea on exertion and a nocturnal fever. Tubercle bacilli were never found in the sputum. Admitted to Brompton Hospital in June 1935.

*Physical examination.* General condition fairly good. Signs of a pneumothorax on the L. side.

*X-ray.* L. pneumothorax, lung completely collapsed.

*Bronchoscopy.* Pus aspirated from L.U.L. bronchus contained fragments of tumour tissue, thought to be carcinoma.

L. pneumonectomy was attempted, but failed owing to dense adhesions; later a course of deep X-ray therapy was given. X-ray examination in October 1936 showed partial re-aeration of the lung.

In May 1940 the patient reported by letter that he was doing his full work, and had no symptoms apart from a slight cough and some dyspnoea on exertion.

*Histology.* The covering epithelium is columnar and rests on an intermediate layer of vascular fibrous tissue which is infiltrated with inflammatory cells and contains large islets of tumour tissue. The tumour proper is composed of solid acini and interlacing columns of cells; there are a few well-formed alveoli. The cells and nuclei are characteristic and no signs of malignancy can be seen. The tumour is thus a bronchial adenoma exhibiting a moderate degree of glandular structure (Group 2).

CASE 13. A woman, aged 66 years, school matron.

*History.* In August 1932 she developed a productive cough and slight dyspnoea on exertion, following a small haemoptysis. Admitted to Brompton Hospital in October 1932.

*Physical examination.* General condition good, fingers not clubbed. Impaired percussion note and numerous rhonchi over R.L.L.

*X-ray.* Collapsed R.M.L.

*Bronchography.* Occlusion of R.M.L. bronchus.

*Bronchoscopy.* Rounded, smooth, white tumour in R. main bronchus arising from R.M.L. bronchus by a pedicle; the growth was removed, but attempts to insert a radon container failed.

The patient remained well and free from symptoms, apart from one attack of broncho-pneumonia on the R. side, until 1938, when she developed a gastric carcinoma which proved fatal.

*Histology.* No epithelium is present and the bulk of the tissue consists of a vascular fibrous stroma containing masses of tumour cells. These are arranged in columns and solid acini with some glandular spaces containing what appear to be desquamated epithelial cells. The tumour cells are uniform in appearance, the majority being polyhedral with well-stained cytoplasm and large oval nuclei which contain a network of chromatin. No mitotic figures or nuclear irregularities are apparent. The general structure is that of a rather undifferentiated bronchial adenoma (Group 2).

CASE 14. A man, aged 32 years, student.

*History.* In 1919 broncho-pneumonia. In 1930 sudden haemoptysis. In 1931 he developed a cough with purulent blood-stained sputum and had occasional pyrexial attacks. These symptoms continued intermittently until October 1936, when he was admitted to St. Thomas's Hospital.

*X-ray.* Collapse of R.L.L.

*Bronchography.* Occlusion of R.L.L. bronchus.

*Bronchoscopy.* Cherry-red vascular tumour in R.L.L. bronchus.

Transferred to Brompton Hospital in December 1936.

*Physical examination.* General condition fairly good, fingers not clubbed. Dullness to percussion and weak air entry over R.L.L. Previous X-ray findings were confirmed and an abscess in the R.L.L. was suspected.

*Bronchoscopy.* Irregular deposit of growth on wall of R.L.L. bronchus; bronchial mucosa was thickened and this thought to be due to adenomatous infiltration.

In May 1937 an abscess in R.L.L. was drained externally; shortly afterwards the patient had a large haemoptysis and then developed a lobar pneumonia in the R.U.L. He died in June 1937.

*Autopsy.* Collapsed R.L.L. containing a large abscess cavity filled with blood clot and also a firm, sharply defined mass of whitish growth extending from the hilum to the base. This entered and obstructed the R.L.L. bronchus, but did not invade the lung tissue; there was bronchiectasis distal to the obstruction. The R.M.L. was also collapsed and the R.U.L. was in a state of grey pneumonic consolidation. The regional lymph nodes showed only inflammatory changes and there were no distant metastases.

*Histology.* That part of the tumour which was removed from the bronchus is covered with columnar epithelium undergoing squamous metaplasia. Beneath this there is a narrow intermediate layer of fibrous tissue containing irregular masses of tumour cells. The tumour proper, obtained at necropsy, is composed of cell masses in a framework of fibrous trabeculae. At the periphery the cells show a tendency to form solid alveoli, but in other parts they lie in irregular masses and columns separated by a loose stroma. The cells are uniform in size, shape, and staining properties, and their large clear nuclei show no mitoses. The general structure is benign, but there is little differentiation and the tumour is therefore classed as Group 2.

CASE 15. A man, aged 49 years, engineer.

*History.* Recurrent small haemoptyses over a period of nine years. No other symptoms apart from an occasional productive cough. Admitted to Brompton Hospital in February 1937.

*Physical examination.* General condition good, fingers not clubbed. No abnormal physical signs in the chest.

*X-ray.* Partial collapse of R.L.L.

*Bronchoscopy.* A red polypoid tumour arising from the wall of the R.L.L. bronchus just below the origin of the M.L. bronchus was removed. A container holding eight 1.5 mc. radon seeds was inserted and was expectorated after 3½ days. In July 1937 a small recurrence was removed and the same amount of radon applied for seven days.

In June 1940 the patient reported that he was well and free from symptoms.

*Histology.* The tumour is covered by thin, squamous epithelium beneath which there is a layer of loose connective tissue which contains numerous blood-vessels and blends with the stroma of the tumour. In the tumour proper, the stroma consists of radiating fibrous trabeculae, between which lie masses of tumour cells arranged in moderately well-formed alveoli and acini, which are separated by basement membranes. The cells and their nuclei are characteristic and the structure is uniform and benign. The general appearance is that of a moderately well-differentiated bronchial adenoma (Group 2).

CASE 16. A married woman, aged 50 years.

*History.* Dry cough for two years which became productive after an attack of 'congestion of the lungs' in December 1936. No haemoptysis. Admitted to Brompton Hospital in July 1937.

*Physical examination.* General condition good, fingers not clubbed. R. side of chest immobile and dull to percussion; breath sounds absent over R. lung, and heart and trachea grossly displaced to the R.

*X-ray.* Complete collapse of R. lung.

*Bronchography.* Occlusion of R. main bronchus.

*Bronchoscopy.* Multiple rounded nodules projecting from the wall of the R. main bronchus; these were removed and a container holding twelve 2 mc. radon seeds was inserted for eight days. No X-ray change was observed.

In June 1940 the patient refused to return to hospital, but reported that she was well apart from a slight, dry cough and some dyspnoea on exertion.

*Histology.* The tumour consists of irregular masses of cells scattered throughout a diffuse, partly hyalinized, fibrous stroma. Some of these cell masses have a central space containing a substance which resembles mucus. The cells are polygonal with scanty cytoplasm and typical nuclei; they show no mitoses or other signs of activity. The general appearance is that of an adenoma showing a fair degree of differentiation, but the wide separation of the clumps of tumour cells is unusual. Jackson and Konzelmann (1937) described a similar case and the tumour is therefore classed as an adenoma Group 2.

CASE 17. A man, aged 36 years, clerk.

*History.* In 1918 influenza followed by a persistent productive cough. In 1919 a pyrexial attack, thought to be due to a lung abscess. In 1920 diagnosed as pulmonary tuberculosis and sent to a sanatorium for nine months. Tubercle bacilli were not found in the sputum. In 1922 a large haemoptysis (1½ pints) followed by blood-stained sputum and persistent pyrexia; sent to another sanatorium for eight months. In 1925 admitted to Brompton Hospital and R. artificial pneumothorax was attempted but failed. Repeated small haemoptyses continued for the next five years. In 1930 again became pyrexial and had a large haemoptysis, the R. phrenic nerve was evulsed at Brompton Hospital. Symptoms continued and he was readmitted in May 1931 after another large haemoptysis.

*Physical examination.* General condition good, fingers not clubbed. Dullness to percussion, absent breath sounds, and a few coarse rales over the R.L.L.

*X-ray.* R. diaphragm paralysed. Collapse of R.L.L.

*Bronchography.* Filling defect in the R. descending bronchus with dilatation of the R.L.L. bronchi.

*Bronchoscopy.* Smooth, round, pink mass projecting into the R. main bronchus; the tumour was treated with diathermy.

One year later, the patient had another large haemoptysis and a R.L.L. lobectomy was attempted, but failed owing to adhesions; diathermy was again applied to the tumour. Patient remained well for three years when he again developed recurrent haemoptyses and dyspnoea. In 1937 bronchoscopy showed a vascular, pedunculated tumour in the R.L.L. bronchus, and a container holding twelve 1.5 mc. radon seeds was inserted for seven days. Three months later the growth was much smaller and six 1 mc. radon seeds were inserted into it. In June 1939 bronchoscopy showed only a scar at the site of the tumour, and the patient had no symptoms.

*Histology.* The tumour is covered by ciliated bronchial epithelium, beneath which lies a dense fibrous capsule. At the periphery there is an irregular band of cells with some inflammatory infiltration, but the bulk of the growth is glandular, showing well-formed alveoli and tubules. The cells are cuboidal or somewhat flattened with clear oval nuclei containing granular chromatin. No mitotic figures or evidence of invasion can be seen, and the general picture is that of a well-differentiated bronchial adenoma (Group 1). This specimen was obtained at the first bronchoscopy.

CASE 18. A man, aged 33 years, decorator.

*History.* In November 1935 pleurisy and pneumonia on the R. side followed by an empyema; the patient refused surgical treatment. During the next year he had a persistent cough and foul sputum. In February 1937 he had a haemoptysis and was admitted to Brompton Hospital.

*Physical examination.* General condition fairly good, gross clubbing of the fingers.

Stony dullness and absence of breath sounds over the base of the R. chest; heart displaced to the R.

*X-ray.* R. basal effusion with underlying collapse of R.L.L.

A sample of fluid from the R. pleural cavity was sterile.

*Bronchography.* Occlusion of the R.L.L. bronchus.

*Bronchoscopy.* Pale, smooth, pedunculated tumour in the R.L.L. bronchus; this was removed and a container holding radon seeds (dose not recorded) was inserted.

Six months later the patient was symptom-free and working. X-ray examination showed almost complete clearing at the R. base and no growth was seen on bronchoscopy. This condition has been maintained, and in May 1940 the patient was well and working.

*Histology.* The covering epithelium is partially stratified. There is an intermediate layer of vascular fibrous tissue which contains numerous islets of tumour cells. The tumour proper consists of a regular arrangement of solid acini supported within a vascular fibrous stroma. The cells are polyhedral and have large, clear nuclei; they show no mitoses or hyperchromatism and there is no evidence of invasion. The appearance is that of a moderately specialized adenoma (Group 2).

CASE 19. A married woman, aged 52 years.

*History.* In 1915 recurrent small haemoptyses for about one year. In 1933 again developed recurrent haemoptyses which usually occurred immediately after menstruation. These continued until 1935 when she developed a persistent cough. In May 1938 pleurisy on R. side, followed by pyrexial attacks and loss of weight. In November 1938 admitted to Brompton Hospital.

*Physical examination.* General condition good, no clubbing of fingers. Dullness to percussion, diminished air-entry, and some rales over R.L.L.

*X-ray.* Collapsed R.L.L.

*Bronchography.* Irregular filling defect in R.L.L. bronchus.

*Bronchoscopy.* Purple, slightly granular, sessile mass arising from wall of R.L.L. bronchus. Eight 2 mc. radon seeds were implanted into the growth, after a portion had been removed for examination.

Three months later another six 2 mc. radon seeds were inserted, and this dose was repeated in February 1940. At this date the patient was very well and had no cough, sputum, or haemoptysis, although there was still some growth visible in the bronchus.

*Histology.* The specimen consists of a portion of the tumour proper. The cells are arranged in masses and columns with occasional small glandular alveoli. The cells themselves are uniform in appearance and show no evidence of malignancy. There is very little stroma and the whole structure is that of an undifferentiated adenoma (Group 2).

CASE 20. A married woman, aged 33 years.

*History.* In 1936 developed slight dry cough. In December 1938 pyrexial attack and increased cough. In March 1939 pyrexial attack lasting 14 days, followed by dyspnoea on exertion and loss of weight. One week later small haemoptysis and pleural pain on L. side. In May 1939 admitted to Brompton Hospital.

*Physical examination.* General condition good, marked clubbing of fingers. Dullness to percussion and distant bronchial breathing over L.L.L.

*X-ray.* Collapsed L.L.L. Well-defined shadow near hilum suggesting an extra-bronchial tumour.

*Bronchography.* Occlusion of L.L.L. bronchus.

*Bronchoscopy.* Rounded, vascular, polypoid mass in L.L.L. bronchus. The bronchial lumen was dilated with bougies and a container holding twelve 1.5 mc. radon seeds was inserted for six days.

In February 1940 symptoms diminished.

*Histology.* The covering epithelium is stratified and rests upon a deep layer of loose connective tissue which is heavily infiltrated with polymorphonuclear leucocytes. At



the periphery the tumour cells are scattered, but in the centre there is a compact glandular mass consisting of alveoli and tubules. Many of the cells are columnar, but the alveoli do not contain mucus. The stroma is unusually plentiful and very vascular. This is a highly differentiated adenoma (Group 1).

CASE 21. A man, aged 35 years, research chemist.

*History.* In 1925 dry pleurisy on R. side. In 1930 pneumonia on R. side, followed by occasional pyrexial attacks with cough and sputum. In 1934 pneumonia on R. side which had a prolonged course. In 1936 recurrence of pyrexial attacks which became more frequent, and in 1937 he commenced to have repeated haemoptyses and became almost bedridden. In July 1939 admitted to Brompton Hospital.

*Physical examination.* General condition good, fingers not clubbed. Impaired percussion note, bronchial breath sounds, and occasional rales over R.L.L.

*X-ray.* Collapsed R.L.L. and R.M.L.

*Bronchography.* R.M.L. bronchus did not fill. Filling defect in R. descending bronchus and bronchiectasis in R.L.L.

*Bronchoscopy.* Large, sessile, vascular mass in R. bronchus at level of M.L. bronchus.

R.L.L. lobectomy was attempted, but failed owing to dense adhesions between the lung and the pericardium. Patient died a few hours later from shock.

*Autopsy.* The tumour in the R. bronchus was projecting from the orifice of the M.L. bronchus. The latter was much dilated and contained the main body of the growth, which was as large as a hen's egg. Part of the tumour extended outside the bronchus, but it was encapsulated and did not invade lung tissue. There were no secondary deposits.

*Histology.* The tumour is enclosed in a fibrous capsule; it is composed of closely packed masses of cells, most of which are typical, but in some areas the nuclei are small and stain darkly. Much of the tissue is degenerate and in some parts it resembles the myxomatous pseudocartilaginous seen in salivary tumours. The cells are arranged in whorls and rosettes resembling glandular elements, and the whole structure is very vascular. A few mitoses are present, and there is definite invasion of the bronchial wall between the cartilages. The general appearance is that of an undifferentiated adenoma (Group 2) which, although completely encapsulated, shows definite evidence of invasion at the site of origin in the bronchial wall.

CASE 22. A woman, aged 22 years, housemaid.

*History.* In March 1939 'pneumonia' on L. side, accompanied by a small, clear pleural effusion. Recovered completely after exhibition of sulphapyridine. In June and August 1939 small haemoptyses. No cough or sputum. In October 1939 admitted to Brompton Hospital.

*Physical examination.* General condition good, fingers not clubbed. Impaired percussion note, weak air entry, and high-pitched bronchial breath sounds over L.L.L.

*X-ray.* Collapsed L.L.L.

*Bronchography.* Occlusion of L.L.L. bronchus.

*Bronchoscopy.* Pink, vascular, fleshy mass arising from medial wall of L.L.L. bronchus at its origin. There was a fullness of the bronchial wall suggesting the presence of an extrabronchial tumour. A container holding nine 2 mc. radon seeds was inserted for seven days. A further bronchogram showed bronchiectasis in the L.L.L. beyond the tumour.

In October 1940 slight attack of pleural pain. Bronchoscopy showed a marked fullness of the bronchial wall, at the apex of which a small, raspberry-like mass projected into the lumen. A L. pneumonectomy is contemplated as there is clearly a large extrabronchial extension of the growth.

*Histology.* The tumour is covered with ciliated, columnar epithelium, beneath which there is a deep layer of vascular connective tissue. The tumour proper is a highly developed, glandular mass, composed almost entirely of tubules in a fibrous framework. There are no signs of malignancy, and the whole structure is that of a well-differentiated adenoma (Group 1).

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FIG. 3. Bronchial adenoma (Case 11). Woman, aged 27. Postero-anterior radiogram showing a rounded opacity in the left middle zone, with partial collapse of the upper lobe

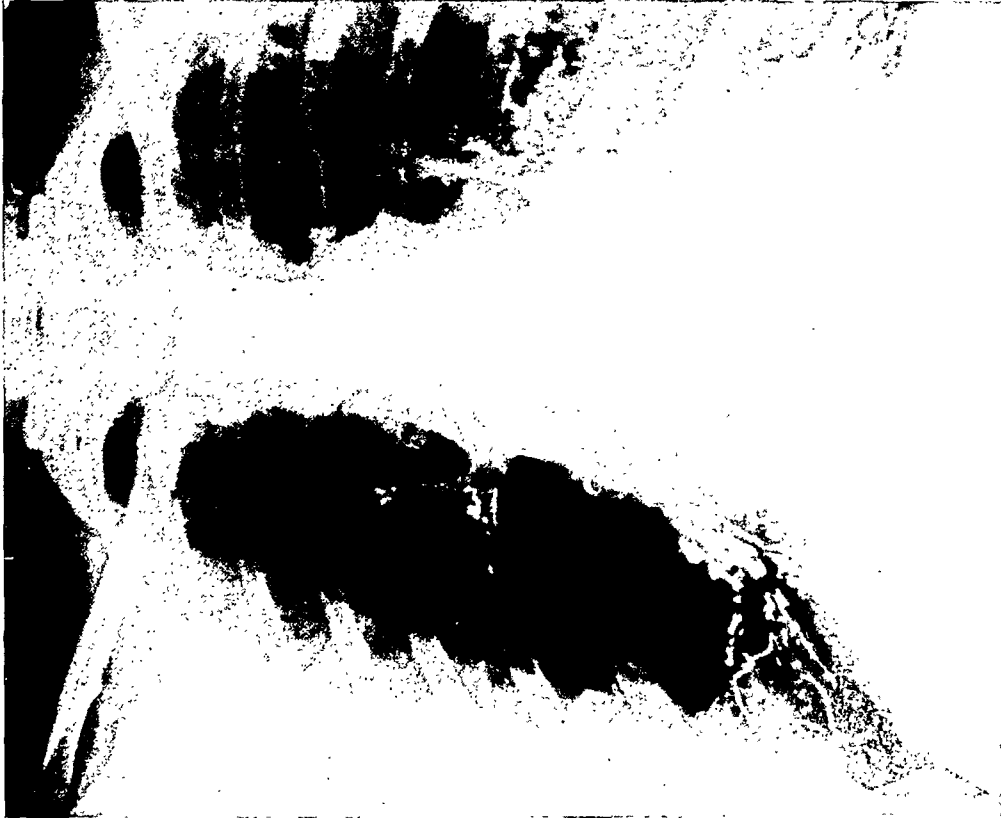


FIG. 4. Bronchial adenoma (Case 7). Woman, aged 34. Bronchogram showing a filling defect in the right lower lobe bronchus, with dilatation of some of the distal bronchi





FIG. 6. Bronchial adenoma (Case 2). Man, aged 47. Photograph of right lung *post mortem*. Showing a polypoid tumour in the main bronchus, with patchy ulceration of its surface

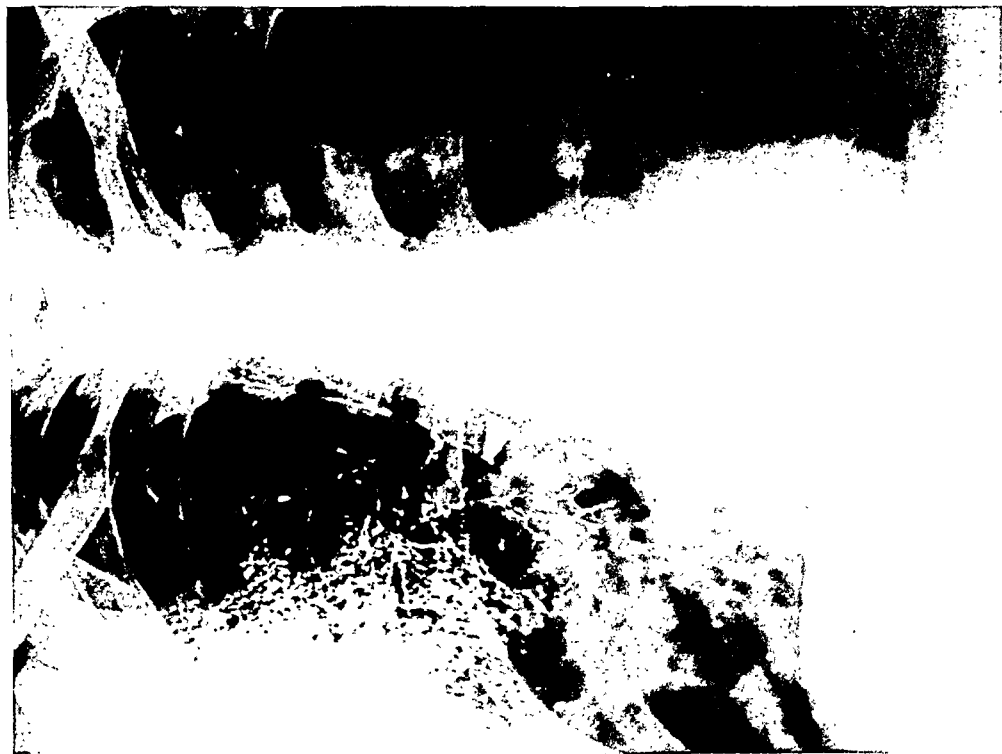


FIG. 5. Bronchial adenoma (Case 5). Man, aged 46. Bronchogram showing complete occlusion of the right lower lobe bronchus. The pool of iodized oil lying in a gutter round the growth makes the bronchus appear to be nipped off sharply



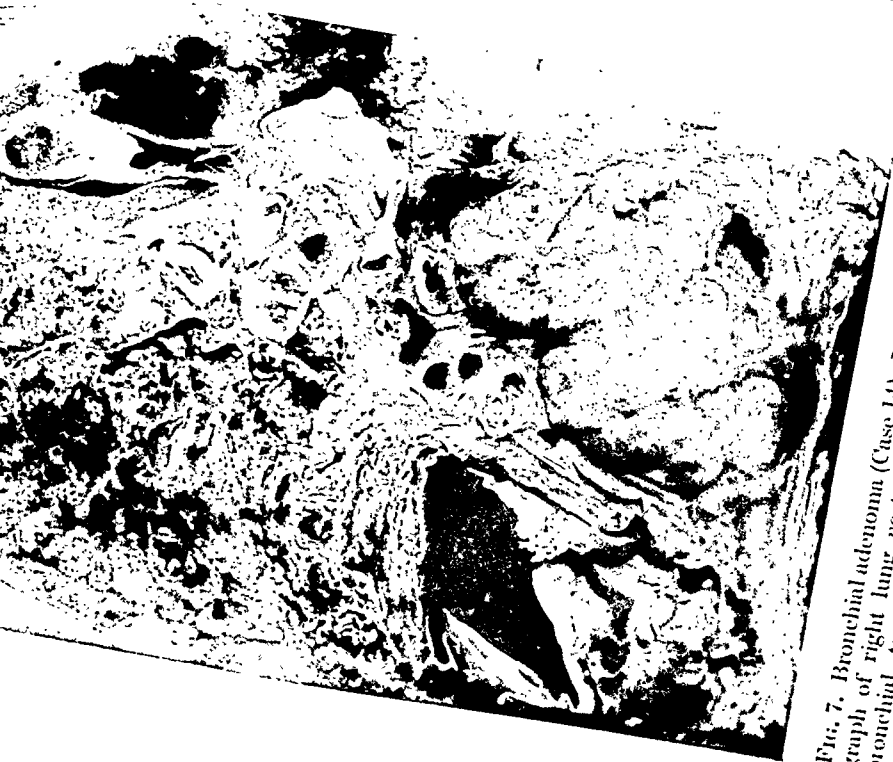


FIG. 7. Bronchial adenoma (Case 14). Man, aged 32. Photograph of right lung *post mortem*. Showing a large extra-bronchial tumour in the lower lobe, compressing the surrounding lung. There is also an abscess cavity filled with blood clot. The upper lobe shows pneumonic consolidation.

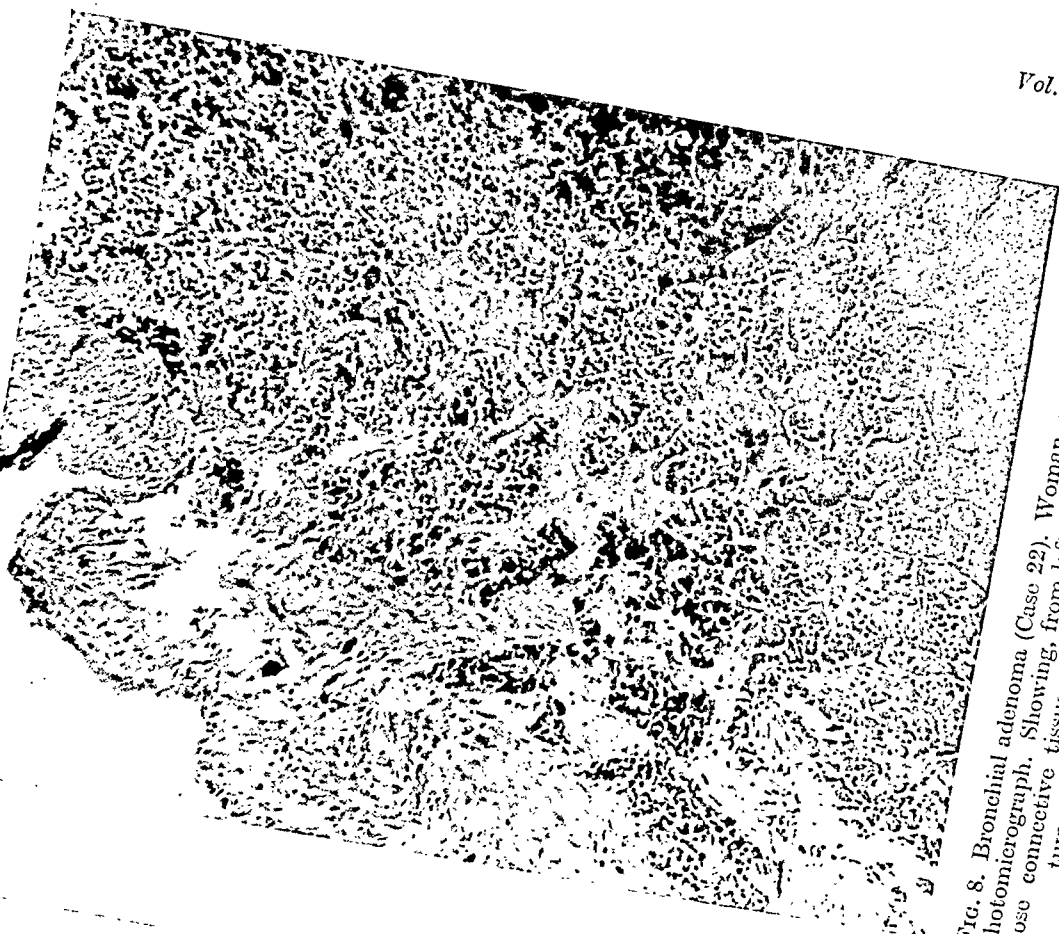


FIG. 8. Bronchial adenoma (Case 22). Woman, aged 22. Low-power photomicrograph. Showing, from left to right, stratified epithelium, loose connective tissue capsule, irregular peripheral parts of the tumour, and regular structure of the tumour proper.





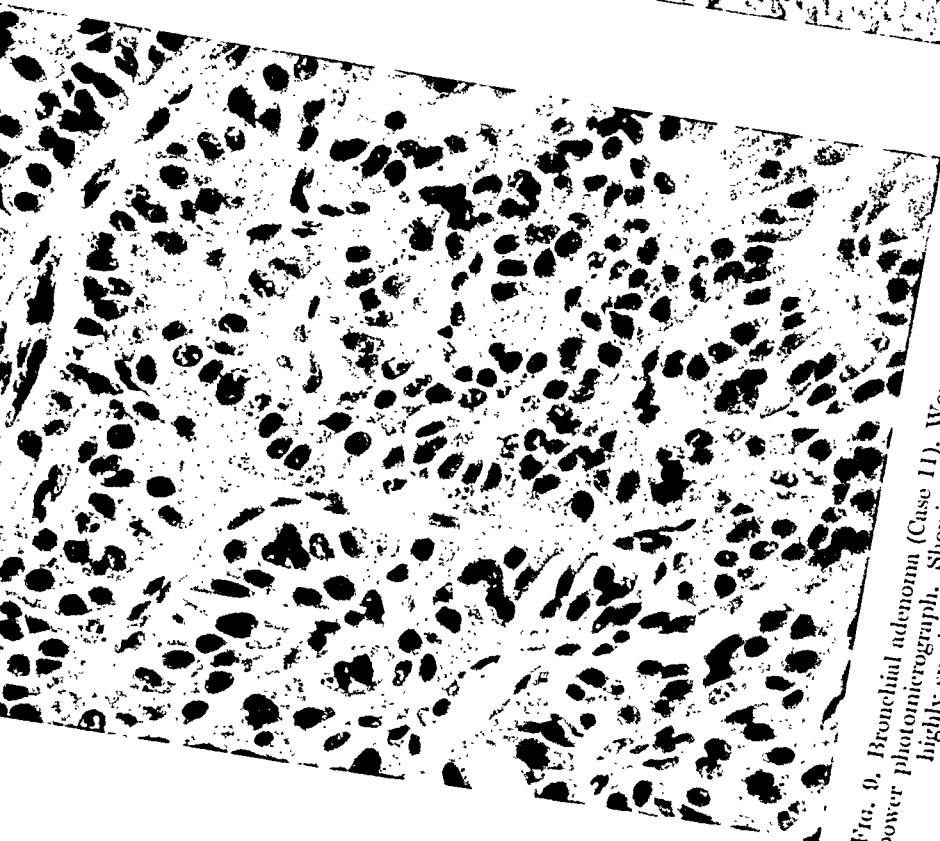


FIG. 9. Bronchial adenoma (Case 11). Woman, aged 27. High-power photomicrograph. Showing the regular structure of the highly specialized type of adenoma (Group 1)

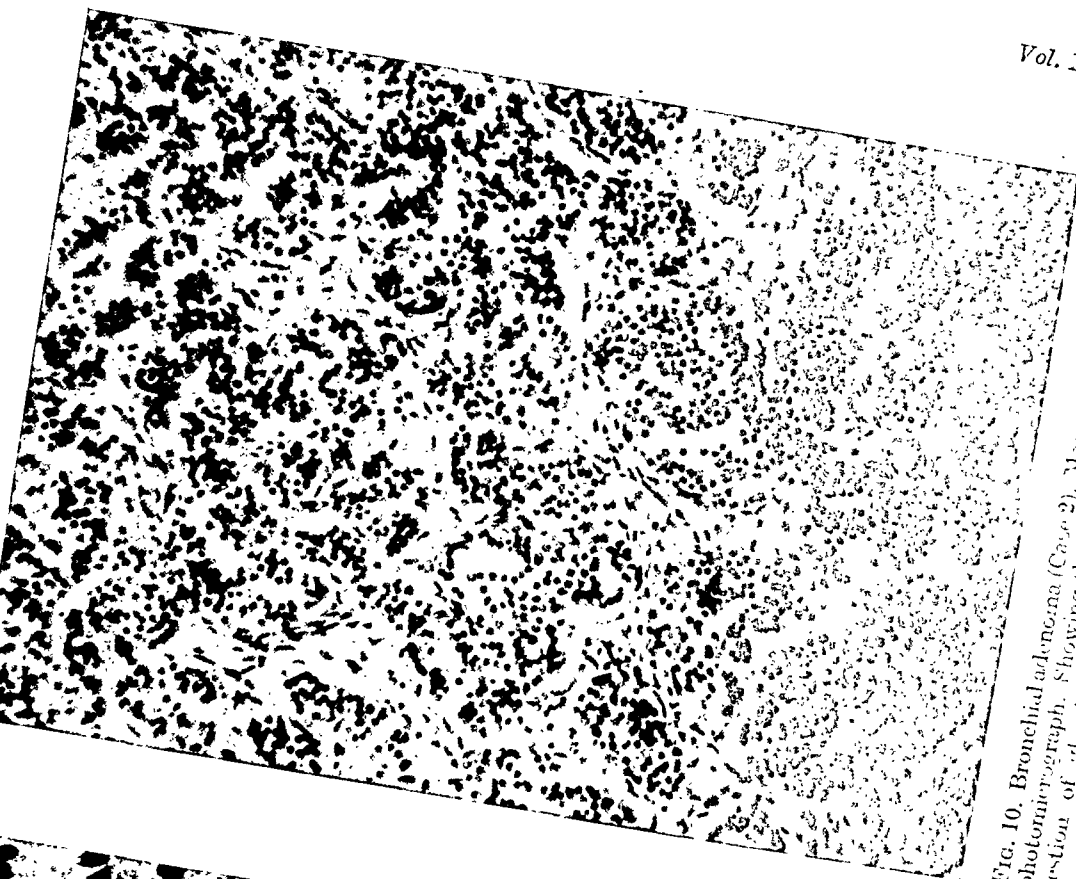


FIG. 10. Bronchial adenoma (Case 2). Man, aged 17. High-power photomicrograph. Showing the looser appearance with a suggestion of glandular structure which characterizes the less differentiated type of adenoma (Group 2)



REFRACTORY ANAEMIA<sup>1</sup>

## I. CLINICAL AND PATHOLOGICAL ASPECTS

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With Plates 13 to 15

1. *Introduction*

THE discovery of the therapeutic value of liver and the more general use of efficient methods of administering iron have brought into prominence a group of patients with severe anaemia whose course cannot be altered by any known treatment. Some of these patients suffer from well defined entities, such as aplastic anaemia or leucopenic leukaemia, but others have conditions which do not appear to fall into any recognized category. Further, the nomenclature that has grown up in connexion with these conditions is confusing, as, for instance, the use of the term 'aplastic anaemia' in cases in which the haemopoietic marrow is not diminished in amount, and of the unfortunate term 'pseudo-aplastic' anaemia.

The disorders reported here are those of patients whose anaemia had failed to yield to any treatment except transfusions of blood. Cases in which the anaemia was found to be secondary to other diseases such as cancer, tuberculosis, lymphogranuloma, nephritis, cirrhosis of the liver, sepsis, infective endocarditis, and frank leukaemia have been excluded. Of the remaining cases of primary anaemia, all have been included in which adequate investigations were made. The series should, therefore, present a fair picture of the varieties of anaemia which fail to yield to treatment, and of the difficulties involved in their classification.

The classification of these cases has presented difficulties, and we have found it impossible to fit many of them into accepted categories. It has therefore seemed wise to put forward a tentative classification which could, at least, include all the cases encountered and avoid obvious terminological inconsistencies. If, in doing so, we have dealt summarily with nomenclature used in previous publications, we must plead that, whereas previous authors have usually described a number of selected cases, we have attempted to bring into some kind of order a large number of cases, of which the only criterion for selection was their failure to respond to any known treatment. If, further, we have seemed dogmatic, this is intentional only in so far as limitations of space have precluded as full a discussion of certain questions

<sup>1</sup> Received November 26, 1940.

as would have been desirable. For the same reason a complete review of previous publications dealing with anaemia which has failed to respond to treatment cannot be attempted. We wish to review certain syndromes, to discuss some points in connexion with aplastic anaemia and leucopenic leukaemia, and to define the terms we shall use in further discussion of the subject.

1. *Aplastic anaemia.* This condition was first described by Ehrlich in 1888. Since then, cases have been recorded by numerous authors (including Smith, 1919; Sheard, 1924; Carey and Taylor, 1931; and Kark, 1937), and there are recent reviews by Lescher and Hubble (1932) and Rosenthal (1938). The essential feature of the condition is a reduction in the amount of haemopoietic marrow below the normal for the patient's age (Turnbull, 1934, 1936). The condition has usually been described as a relatively acute one, almost confined to children and young adults, and characterized by severe anaemia and haemorrhages. The blood picture was described as showing a decrease of erythrocytes, granular leucocytes, and platelets, with erythrocytes normal in size and shape, and with an absence both of signs of regeneration and of immature cells in the blood. More recent accounts have shown that the disease may occur at other ages, that it may have a more variable course than was previously recognized, and that the presence in the blood of slight macrocytosis, of immature red and white cells in small numbers, and of an increased percentage of reticulocytes is compatible with the existence of severely hypoplastic marrow at autopsy. The term 'aplastic anaemia' can reasonably be used to include any anaemia in which there is definite hypoplasia of the haemopoietic marrow, but if the meaning of terms is to be respected, it cannot be used to describe cases of anaemia, however similar the clinical picture may be, if the marrow is not hypoplastic.

2. '*Pseudo-aplastic anaemia.*' Occasional cases clinically simulating aplastic anaemia, but later found to have cellular marrow, have been recorded from time to time, and various names such as pseudo-aplastic anaemia and progressive hypocythaemia have been suggested for them. Thompson, Richter, and Edsall (1934) reviewed this subject and described 13 cases in which the original clinical diagnosis was aplastic anaemia. The bone marrow, however, studied in sections obtained at autopsy or biopsy, was found to be hypoplastic in three, normal in cellularity in two, moderately hyperplastic in two, and considerably hyperplastic in the remaining six. No post-mortem evidence of leukaemia was found in any case. This statement appears open to question in their Case 10, one of benzol poisoning, later reported in detail by Andersen (1934), in which the liver and other organs were infiltrated with oxydase-positive cells; it seems probable that this was a case of leucopenic myeloid leukaemia. In nine others the clinical findings suggested aplastic anaemia, but the bone marrow was either normal in cellularity or hyperplastic. Rhoads and Miller (1938) described the histology of the marrow in 69 cases of anaemia refractory to treatment, and confirmed and amplified the conclusions of Thompson and his co-workers.

3. *Achrestic anaemia.* Israëls and Wilkinson (1936) described a syndrome,

of which the main features were a megalocytic anaemia, a normal gastric acid secretion, incomplete or temporary response to liver therapy, megaloblastic hyperplasia of the bone marrow, a prolonged course, and a fatal termination. In spite of criticism (Castle and Minot, 1936; Zanaty, 1937; Mogensen, 1938) the authors have vigorously defended their syndrome as a specific one, and have objected to its inclusion in the group of 'pseudo-aplastic anaemias' (Israëls and Wilkinson, 1938, 1940). Many of our cases showed some macrocytosis, a normal gastric acidity, and a fatal termination, while several of them had a history of a favourable response to liver extract at some stage in their disease. Several, also, had hyperplastic marrow with a histological picture indistinguishable, in our opinion, from that illustrated by Israëls and Wilkinson (1936). We are inclined to believe that achrestic anaemia is not a specific syndrome. If, however, the term 'achrestic anaemia' is used, it should be reserved strictly for cases which fulfil all the criteria laid down by its authors. The term 'achrestic-aplastic group', which has recently appeared in publications, can only bring confusion.

4. *L'anaémie maligne intermédiaire*. Chevallier (1936) described three cases of his own with two from recent French literature, and considered that they represented a syndrome intermediate between pernicious and aplastic anaemia. The anaemia was usually hyperchromic and macrocytic, and failed to respond to liver therapy, though some spontaneous ameliorations occurred. The author considered that in some instances regeneration and in others aplasia of the marrow predominated. One patient was examined *post mortem*, and the marrow was severely hypoplastic. These cases do not appear to differ significantly from those previously described as aplastic or 'pseudo-aplastic' anaemia, and seem to belong to the group under discussion.

5. *Medullary lymphadenosis or medullary lymphadenoid pseudo-leukaemia*. Occasional cases have been described of a condition which clinically simulated aplastic anaemia, but was considered *post mortem* to be leucopenic lymphatic leukaemia, in which the leukaemic process was confined, or almost confined, to the bone marrow. The most recent examples are those of Zanaty (1934), but it is open to question whether the cases described under this heading were really cases of leukaemia.

6. *Leucopenic leukaemia*. The nomenclature of this and related diseases has become even more confusing than the diseases themselves, probably because in most instances the terms used have never been clearly defined. Forkner (1938) lists 23 names that have been given by different authors to cases of this type, and the list is not a complete one. All leukaemia, as suggested by Forkner, can first logically be divided into acute and chronic forms. It can then be divided into two types according to whether an increased number of leucocytes is or is not present in the peripheral blood stream. The terms leukaemia and leucocythaemia, originally used to describe the condition of the blood, appear now to be firmly established as the terms for the disease process, that is, for the presence of leukaemic tissue in the bone marrow and other organs. Attempts to substitute other terms such as

leukosis, myelosis, or lymphadenosis for the disease process seem doomed to failure. If leukaemia is used for the disease process, it is illogical to use the terms 'leukaemic' and 'aleukaemic' to indicate the presence or absence of an increased number of leucocytes in the blood. The substitution of the terms 'leukaemic' and 'subleukaemic' (Forkner, 1938) does not seem to solve the difficulty. Leukocythaemic and aleukocythaemic leukaemia, as suggested by King (1917), are logical but cumbersome. The simplest solution, one which is not new and does not involve the introduction of unfamiliar terms, seems to be to use the term 'leukaemia' for the disease process in its usual form with an increase of white cells in the blood, and 'leucopenic leukaemia' for the form without such an increase; we shall use these terms in this sense. For the purpose of this study it is important to have as definite a picture of leucopenic leukaemia as possible, since it is likely that cases of incurable anaemia with hyperplastic marrow have often been diagnosed falsely as leucopenic leukaemia.

The myeloid form of leucopenic leukaemia appears to be commoner than the lymphatic type and this condition was first clearly described by Hirschfeld in 1914 under the name aleukaemic myelosis. Hirschfeld (1925) reviewed the subject and described the condition as pathologically similar to myeloid leukaemia, except for the absence of an increased number of leucocytes in the peripheral blood. The main features of the disease were enlargement of the liver and spleen and usually also of lymphatic glands during life, and great proliferation of myeloid tissue in the bone marrow, and in the liver and spleen at least *post mortem*. There might or might not be myelocytes in the peripheral blood. Jaffé (1927), Pinkerton (1929), Weber (1932), Baldridge and Fowler (1933), Mettier and Purviance (1937), Scott (1939 *a*), and Hynes (1940), among others, have described cases of leucopenic leukaemia, and the whole subject is discussed in Forkner's (1938) monograph.

Leucopenic leukaemia is a rare disease in any form. In all the cases of the chronic myeloid form that we have seen reported, in which the diagnosis was proved *post mortem*, there has been enlargement of the spleen and usually also of the liver and lymphatic glands at some period during life, and extensive myeloid infiltrations outside the bone marrow at autopsy. Similarly in the great majority, if not in all, of the proved cases of the chronic lymphatic form there has been enlargement of at least some lymphatic glands clinically, and lymphocytic infiltrations pathologically. We have found no evidence for the occurrence of a purely medullary form of chronic myeloid leukaemia, and cases of chronic medullary lymphatic leukaemia, if they occur at all, do so exceedingly infrequently. It seems, therefore, that cases of chronic anaemia with leucopenia and a few immature cells in the peripheral blood should not be diagnosed as leucopenic leukaemia in the absence of other evidence of that condition. For the purpose of this report we have considered the finding of some leukaemic infiltration outside the bone marrow necessary for a proved post-mortem diagnosis of chronic leucopenic leukaemia. This may appear arbitrary, but it seems likely to lead to fewer errors in

diagnosis than does the absence of any definite limit to what may be diagnosed as leukaemia.

There are serious objections to all the terms that have been used to describe the kind of anaemia under discussion. 'Aplastic anaemia' and 'panmyelophthisis' cannot be used for the whole group of cases because the haemopoietic marrow is often increased in amount. The term 'pseudo-aplastic anaemia' can lead only to confusion. 'Pluricytopenia' cannot logically be used for cases without granulocytopenia or thrombocytopenia, and such cases are not uncommon; the term 'progressive hypocythaemia', or better 'progressive oligocythaemia', ignores the important fact that remissions are not uncommon. Until a nomenclature based on aetiology becomes possible it seems best to refer to the whole group of cases by a name which reflects its most significant characteristic—failure to respond favourably to the exhibition of substances which cure the great majority of cases of anaemia. Further subdivision according to the morphological characteristics of the peripheral blood would lead to a complicated classification of doubtful utility. Examination of the bone marrow by sternal puncture or biopsy is important in these cases for diagnosis and prognosis, and seems to provide the best basis for further subdivision.

We therefore refer to the whole group as 'primary refractory anaemia'.<sup>2</sup> We use 'refractory anaemia with hypocellular marrow' as synonymous with 'aplastic anaemia', and 'refractory anaemia with normal' or 'hypercellular marrow', as the case may be, for conditions which have been referred to by such names as pseudo-aplastic anaemia and pluricytopenia. By 'leukaemia' we mean the usual form of that disease in which there is, in addition to changes in the bone marrow and other organs, an increased number of white cells in the peripheral blood. By 'leucopenic leukaemia' we mean conditions which are similar to leukaemia in every respect, except for the absence of an increased number of white cells in the peripheral blood stream.

## 2. *Histology of the Bone Marrow in Refractory Anaemia*

The histology of the bone marrow in many of the cases in this series has already been described by Rhoads and Miller (1938). Since their paper appeared, a number of cases has been added to the collection, bringing the number with biopsy sections available to 50, with post-mortem sections available to 25, and with both biopsy and post-mortem sections available to 22.

Marrow was removed from the sternum during life by the technique of Rhoads and Castle (1933). At autopsy marrow was usually removed from the sternum, a rib, a lumbar vertebra, and a femur. In all instances the marrow was fixed in Zenker's fluid containing 5 per cent. of acetic acid, dehydrated and cleared by the usual methods, and embedded in paraffin.

<sup>2</sup> The term 'refractory', as meaning, of a disease, unyielding to treatment, has the sanction of long usage. Robert Boyle (1671), in *Some considerations touching the usefulness of Experimental Naturall Philosophy, The Second Tome*, writes of 'divers stubborn Disease, that had been found refractory to all ordinary Remedies'.

Decalcification was not attempted. Sections were stained with eosin and methylene blue. In addition, in many instances marrow was fixed in Zenker's fluid prepared with formaldehyde (U.S.P. 1:10) in place of acetic acid, and stained by the Giemsa method. The first method has the advantage for routine purposes that sections are obtained which have uniformly good nuclear staining, and fade only slowly. It has the disadvantage that the cytoplasm of early erythrocyte precursors remains almost unstained, and the granules in the cytoplasm of neutrophil polymorphonuclears are often poorly defined.

The terminology used is that of Turnbull (1934, 1936). Thus it is assumed that there is a pluripotential stem cell, the haemocytoblast, which is derived from the reticulo-endothelium and gives rise to the precursors of erythrocytes, granular leucocytes, and lymphocytes. In the development of erythrocytes there is formed from haemocytoblasts a series of primary erythroblasts, in which the size of the cell diminishes and the nucleus becomes denser as the cell becomes more mature. In normal post-foetal erythropoiesis, at about the same time as the nucleus loses its pattern, the cytoplasm, at first basophil, becomes polychromatic and then eosinophil, and a mature normoblast results. Similarly, in the formation of granular leucocytes haemocytoblasts give rise to myelocytes, and myelocytes to leucocytes. The appearances of these cells described by Turnbull (1934, 1936) are those seen in sections fixed in formalin and stained with Jenner's stain by the method described by him (1931). In sections fixed in Zenker's fluid containing acetic acid, and stained with eosin and methylene blue, the appearances, particularly of haemocytoblasts and primary erythroblasts, are different. By this method haemocytoblasts, which can be seen in conspicuous groups in the marrow of any case of pernicious anaemia in relapse, are large cells with large round or slightly oval nuclei. The nuclei are pale with a few threads of chromatin and a few nucleoli; the narrow rim of cytoplasm is ill defined and stains a pale greyish-blue. The earlier primary erythroblasts are smaller cells with a little more chromatin and a few nodes in the nuclei; the cytoplasm is again ill defined and pale greyish-blue. These cells may closely resemble medium-sized lymphocytes. The later primary erythroblasts and early normoblasts have denser nuclei, often with a somewhat 'clock-face' pattern and darker basophil or polychromatic cytoplasm. Mature normoblasts have pyknotic nuclei and eosinophil cytoplasm. The granules of neutrophil myelocytes, though indistinct, stain a dull pink and can usually be distinguished by careful focusing.

The histology of marrow sections has been described mainly under six headings:

1. *The degree of cellularity.* Since more biopsy than autopsy material was available, the question arises how far it is possible to estimate the cellularity of the marrow as a whole by the appearance of one or two sections of sternal tissue. In the examination of post-mortem material from adults, it was found that satisfactory sections of marrow from the sternum, a rib, and



a lumbar vertebra were usually approximately uniform in cellularity. The femoral marrow was frequently much less cellular than that from other sites. A conspicuous difference in the degree of cellularity of the sternal, costal, and vertebral marrow was present in only one case, and this patient had recently had X-ray treatment, a circumstance which may have accounted for the differences observed. It seems, therefore, that the appearance of a satisfactory section of the sternal marrow gives an approximate indication of the degree of cellularity of marrow in the bones of the trunk. It does not give any indication of the anatomical extent of the haemopoietic marrow in the extremities. We have as far as possible used the terms hyperplastic, hypoplastic, and aplastic to refer to the anatomical extent of the haemopoietic marrow, compared with the extent which is normal for the patient's age; and we have used the terms hypercellular and hypocellular to indicate deviations from a normal proportion between the amounts of fat and haemopoietic cells, as estimated under the microscope. Such estimates of cellularity in sections can be regarded only as significant if the deviation from the normal proportion is considerable.

2. *Focal or diffuse distribution.* In many sections, and much more frequently in those from biopsy than from autopsy, the distribution of haemopoietic marrow was patchy, with intervening areas of haemorrhage, particularly in formalin-fixed sections, or of a curious eosinophil fibrillar material, particularly in sections fixed by acetic acid. These latter are referred to in descriptions as 'pink structureless areas'. We are unable to say whether these appearances were due to trauma in the course of removal of marrow or whether they indicate the existence of a process of active damage of the marrow during life. The matter requires further investigation before the appearances can be interpreted.

3. *Architecture.* This is described as normal or atypical; normal when the arrangement of cells in strands and groups between fat cells is preserved, and atypical when this is replaced by a diffuse arrangement of the haemopoietic cells, whether these are arranged densely or sparsely.

4. *Predominant series of cells.* Where maturation is not seriously disturbed it is possible to detect any considerable deviation from the normal ratio of approximately one-third nucleated erythropoietic to two-thirds leucopoietic cells. This estimate is independent of the cellularity of the marrow as a whole. Thus a marrow may be hypocellular, but there may still be a relative increase in the proportion of erythropoietic to leucopoietic cells. Such a marrow is described as 'hypocellular with a relative predominance of erythropoiesis'.

5. *Maturity.* In normal marrow there is a predominance of the more mature forms in each cell series; that is, of polymorphonuclear leucocytes, normoblasts, and erythrocytes. By immaturity of the marrow is meant a relative increase in the proportion of less mature forms. Thus in a very immature marrow there may be a few normoblasts and few or no polymorphonuclear leucocytes, but numerous haemocytoblasts and early

erythropoietic and leucopoietic cells. The degree of immaturity may be different in the erythropoietic series of cells. We use these terms in a descriptive sense only, and without subscribing to the theory of 'maturation arrest' sometimes advanced to explain the appearances.

6. *Identification of individual cells.* This presents no difficulty in the case of haemocytoblasts, eosinophil normoblasts, myelocytes, and polymorphonuclear leucocytes. In the refractory anaemia reported here, however, one of the most frequent findings was an increase in medium-sized and small basophil staining cells. The identification of these cells, particularly where the normal maturation is seriously disturbed, presents difficulties which are discussed here. These cells do not have a uniform size or appearance. In size they vary from about that of medium-sized to small lymphocytes. Many of them in stained sections are indistinguishable from lymphocytes. Others, both from their appearance and their position (in many instances in maturing erythropoietic islands) can definitely be identified as primary erythroblasts and basophil normoblasts. Still others have eccentrically placed nuclei with a 'clock-face' arrangement of the chromatin chiefly at the periphery, and basophil cytoplasm; these resemble plasma cells. This whole group of cells appears to correspond to those described by Selling (1916) in his classical description of experimental benzol poisoning as 'lymphocytes and polyblasts'. There seem to be three main possibilities as to the nature of these cells:

(a) They may be lymphocytes. Various authors have described great increases in the percentage of lymphocytes in smears of the marrow in this condition. Scott (1939 *b*) recently reported up to 85 per cent. of lymphocytes in the bone marrow of a series of cases of aplastic anaemia. Jordan (1939) has reported a case where the cells appear to be almost entirely of the type under discussion, and claimed that the histological appearances of the marrow and lymphoid organs support the theory that under conditions of emergency mature lymphocytes migrate from the lymphoid organs to the bone marrow and there serve as erythroblasts.

(b) Rhoads and Miller (1938) described these cells as the 'primitive cells' of Sabin, on the basis of supravital studies of smears from portions of marrow taken at biopsy. Sabin, Miller, Smithburn, Thomas, and Hummel (1936) described the primitive cell in the marrow of foetal and young growing rabbits as the earliest precursor of all types of leucocytes in a polyphyletic scheme of haemopoiesis. These cells can be distinguished from lymphocytes in supravital preparations, but in stained preparations they are practically indistinguishable, differing from lymphocytes only, it is said, in having less chromatin in the nucleus. Rhoads and Miller (1938) described up to 90 per cent. of these cells in marrow from cases of refractory anaemia. They included, however, under the term primitive cells, cells having a 'somewhat denser arrangement of the nuclear chromatin than do lymphocytes'. From an examination of sections of the marrows in which these large percentages of primitives were described, it seems that at least some of the cells referred to as having a denser arrangement of the nuclear chromatin than do

lymphocytes, were later members of the series of primary erythroblasts and basophil normoblasts.

(c) The preceding theories have been based mainly on the percentage counts of cells in smears and supravital preparations. The examination of stained sections has the advantage that the arrangement of cells gives an indication of their position in maturation. Thus, in sections showing large numbers of the type of cells under discussion, it is possible to find many which appear indistinguishable from lymphocytes and therefore by definition from the 'primitives' of Sabin. In many cases, though not quite in all, these cells are found in juxtaposition, sometimes with haemocytoblasts and sometimes with unmistakable late primary erythroblasts and normoblasts. This suggests that these cells are in fact erythroblasts. Three other facts support this interpretation: firstly, in these marrows unmistakable lymphocytes occur quite often in typical lymph follicles, and the arrangement of the cells is then quite different from that of the cells in marrows consisting almost entirely of the lymphocyte-like cells under discussion. Secondly, in marrows consisting almost entirely of these cells, the only more mature cells found are normoblasts. Thirdly, it is noticeable in cases where the marrow at biopsy consists almost entirely of cells of this type that anaemia is usually no more than moderate, whereas granulocytopenia is profound.

We therefore believe that the group of medium-sized and small basophil staining cells, which are conspicuously increased in numbers in the marrow in many cases of refractory anaemia, consists of primary erythroblasts, basophil normoblasts, and some atypical erythropoietic cells. We leave open the question whether actual lymphocytes or the cells described by Sabin (1936) as primitives can function as erythroblasts under pathological conditions. Occasionally small cells of this type with pale nuclei have been seen with a few neutrophil or eosinophil granules in their cytoplasm, apparently developing into myelocytes, as described and illustrated by Sabin, Miller, Smithburn, Thomas, and Hummel (1936). It is possible that these cells are in fact atypical small haemocytoblasts.

Rohr and Hafter (1937) have criticized the examination of post-mortem sections of marrow, and claim to have shown that changes in the appearance of marrow cells take place so rapidly after death that such examinations are valueless. We have examined from this point of view 21 of our cases in which the interval between death and the beginning of the autopsy is recorded. The average interval in these cases was 5.6 hrs. The autopsy in two was begun within 30 min. of death and in six the interval was 9 hrs. or longer. There was some difference in the appearance of cells in cases examined soon and late after death, but we were unable to confirm the conclusion of Rohr and Hafter that disfiguring changes occur rapidly enough to render useless the examination of post-mortem marrow. It is possible that the extreme changes described by these authors might have occurred in the absence of adequate refrigeration; it is also possible that the changes observed by them were due to their use of the technique of sternal puncture,

involving the exposure of cells undergoing post-mortem degeneration to severe changes of pressure. Further, we have ourselves noticed that smear preparations from post-mortem marrow are usually unsuccessful.

*Classification of marrow changes in refractory anaemia.* The appearance of the marrow in our series varied considerably. It is possible to pick out cases belonging to distinct types, as has already been done by Rhoads and Miller (1938), and these types of marrow are found to be correlated to some extent with the clinical findings and course. The types are not sharply defined, some cases appear to be intermediate between two types, and in certain cases one type of marrow was found at biopsy and another at autopsy. Though it is useful, particularly for purposes of prognosis, to attempt to define these types, and it is possible that they may be found to bear some relation to differences in aetiology, the similarity of cases of refractory anaemia as a whole appears more significant than the differences between individual cases and groups of cases. Too great significance should not, therefore, be attached to a classification into types. The types are as follows:

1. Refractory anaemia with partly mature cellular marrow (Plate 14, Fig. 4, E and F). Marrow taken at biopsy was available in 23 cases of this type, and autopsy marrow was available in five. The degree of cellularity varied from a proportion of fat to haemopoietic cells that appeared almost normal to a densely cellular marrow with no fat cells visible. Under low magnification this type of marrow was readily distinguished from the hypocellular type by the degree of cellularity and from the immature cellular type by the presence of considerable but varying numbers of eosinophil cells, that is, myelocytes, polymorphonuclear leucocytes, and erythrocytes.

Under higher magnification the cell content varied somewhat in different cases, but showed certain distinguishing characteristics. The proportion of erythropoietic to leucopoietic cells appeared considerably increased above the normal in all but a few cases. There was, however, in every case a considerable number of myelocytes and polymorphonuclear leucocytes, the proportion of myelocytes being increased. In a few cases eosinophil myelocytes were present in considerable numbers. In all cases the proportion of immature erythropoietic cells to normoblasts was greatly increased. In several cases there were numerous haemocytoblasts, often in conspicuous groups, and the marrow in these cases appeared to us indistinguishable from that described and illustrated by Israëls and Wilkinson (1936) as the marrow of achrestic anaemia. In the two cases of moderate anaemia attributed to benzol poisoning, in which prompt remissions occurred, there were fewer immature erythropoietic cells, and greater numbers of normoblasts, mostly with eosinophil cytoplasm. In the largest group of cases the picture appeared intermediate between these two varieties, the predominant cells being primary erythroblasts and the lymphocyte-like cells which we believe to be erythroblasts. In several cases there were also numerous large basophil normoblasts, sometimes arranged in conspicuous groups. In a few instances with very distinctly hyperplastic marrow megakaryocytes were present in considerably

greater numbers than normal, in other cases they were present in about normal numbers, in reduced numbers, or were absent. Phagocytes containing hemosiderin were present in small numbers in several cases and in considerable numbers in two.

The term 'partly mature' has been applied to this type of marrow to distinguish it from those types in which more conspicuous disturbances of maturation are present. Of the four types of marrow described this type differs least from normal. Though it is immature, some maturation to the stage of normoblast and of polymorphonuclear leucocytes is present, and megakaryocytes are usually to be seen. Correspondingly in cases of this type the clinical course is often prolonged, leucopenia and thrombocytopenia may be slight or absent, and remissions occur more frequently than in any other type.

2. Refractory anaemia with hypocellular marrow, or aplastic anaemia (Plate 13, Fig. 3, c and d). Marrow taken at biopsy was available in nine cases of this type. Three of them were also examined *post mortem* and post-mortem marrow was available in four other cases, giving seven in all. Only cases in which the marrow was unmistakably hypocellular have been included in this group. Thus in all of them the proportion of fat cells to haemopoietic cells was greatly increased. The number of haemopoietic cells varied. In two cases there were not more than a few groups of two or three small basophil cells to be found in a whole section of sternal marrow. In several cases there were strands and small groups of haemopoietic cells, mostly erythropoietic cells, primary erythroblasts, lymphocyte-like cells, and relatively few normoblasts, almost all with basophil cytoplasm; occasional myelocytes, almost all eosinophil, and occasional polymorphonuclear leucocytes were seen. No megakaryocytes were seen in any of the sections from cases in this group. In several cases the distribution was patchy, and small areas of very actively haemopoietic marrow were scattered in otherwise distinctly hypocellular marrow. In two cases of moderate anaemia attributed to benzol poisoning in which a prompt remission occurred, the marrow, though hypocellular, differed from those described above in that it was more mature with a greater proportion of normoblasts, usually with eosinophil cytoplasm, and fewer early erythropoietic cells. In two cases haemosiderin was present in varying amounts.

Excluding the two cases with mild anaemia attributed to benzol poisoning in which a prompt remission occurred, seven cases were seen with hypoplasia of the sternal marrow at biopsy. In two with the most severe hypoplasia seen (Cases 17 and 24), and in one with moderate hypoplasia (Case 18), remissions occurred and are known to have lasted several years. The remaining four cases proved fatal.

3. Refractory anaemia with immature cellular marrow, or chronic granulocytopenia (Plate 13, Fig. 3, e and f). Marrow taken at biopsy was available in seven cases of this type. Six of these were also examined *post mortem*, and post-mortem marrow was available in fourteen cases in all. The

cellularity of the marrow in these cases varied. In a few biopsy specimens the marrow was densely cellular, and there was little or no sign of normal marrow architecture, in most cases the marrow was distinctly but not densely hypercellular, in some cases normal architecture was preserved, but in others the cells were arranged diffusely. In one case the marrow *post mortem* was hypocellular, but the cells were arranged diffusely with almost no sign of normal architecture.

Under low magnification marrow of this type is seen to be composed almost entirely of basophil-staining cells with the dark nuclei of normoblasts standing out conspicuously, either singly or in groups. The cells, however, vary in size and have neither as uniform an appearance nor as dense an arrangement as have the cells in lymphatic leukaemia (Plate 14, Fig. 4, A and B).

Under higher magnification the appearance of the marrow in different cases differed slightly, according to which type of cell predominated. In some cases there were numerous haemocytoblasts and early primary erythroblasts with few late primary erythroblasts and normoblasts, the latter having basophil cytoplasm or appearing as pyknotic nuclei with no recognizable cytoplasm. In other cases there were large numbers of smaller cells, some resembling lymphocytes, some resembling plasma cells, and some being undoubted primary erythroblasts. The reasons for regarding most, if not all these cells, as erythroblasts have already been discussed. In these latter cases too there was a relatively small number of normoblasts, usually with basophil cytoplasm. Scattered among these basophil cells were myelocytes, never more than a few, and usually extremely few. They were almost invariably eosinophil myelocytes. An occasional polymorphonuclear leucocyte could usually be found by searching several fields, but sometimes these cells appeared to be completely absent. Erythrocytes were few in number, and it was presumably the great scarcity of haemoglobinated cells, erythrocytes and normoblasts, that accounted for the fact that this type of marrow, though hypercellular, might appear quite yellow to the naked eye, and give the impression of aplasia. Megakaryocytes were either present in reduced numbers or were absent. Phagocytes containing haemosiderin were present in small numbers in some cases.

It will be evident that in cases in which there was a great predominance of lymphocyte-like cells in the marrow, the appearance was very similar to that of lymphatic leukaemia. Frank (1915), discussing this point, said that lymphocytic infiltration might occur in the marrow of aplastic anaemia, especially that due to benzol poisoning, and when the infiltration was considerable, there was no clear distinction between aplastic anaemia and medullary lymphadenoid pseudo-leukaemia. We have already questioned the existence of a purely medullary lymphadenoid leukaemia, and it seems likely that the cases described as such should be regarded not as cases of leukaemia, but as examples of refractory anaemia of the type described in this section. Marrow qualitatively of this type has been seen in several cases

of acute agranulocytosis (Plate 15, Fig. 5, E and F), and the marrow described and illustrated by Martland (1931) in the earlier stages of radium poisoning appears indistinguishable from that seen in some of our cases. We believe that these cases represent a chronic condition related to acute agranulocytosis. Case 34 of the present report, in which there was clinical evidence of sensitivity of the leucopoietic system to allonal, had marrow of this type at biopsy and appears to illustrate the relationship between the two conditions. Excluding this last case, the course of the disease in this group was relatively short, was associated with considerable fever and often with necrotic ulceration of the mouth, pharynx, or peri-anal regions; the outcome was uniformly fatal.

4. Refractory anaemia with fibrosis, sclerosis, and giant cell hyperplasia of the marrow, or myelosclerosis (Plate 15, Fig. 5, A, B, C, and D). Marrow taken at biopsy was available in four cases of this type and two were examined *post mortem*. These cases appear to be examples of the condition or group of conditions first described in English by Donhauser (1908) in recording a 'case of splenomegaly with sclerosis of the bone marrow'. Such cases are rare, but recent examples have been described by Hewer (1937), Tudhope (1937), Hickling (1937), Mettier and Rusk (1937), and Bamforth and Kendall (1939), and the condition is reviewed by Chapman (1933) and Rosenthal (1938). Its characteristic features are splenomegaly, anaemia, fibrosis or sclerosis of the marrow, and extensive extramedullary haemopoiesis, the haemopoietic tissue usually containing conspicuous numbers of megakaryocytes. The condition has been described as osteosclerosis, as myelosclerosis, and when the presence of megakaryocytes in the tissues has been the most prominent feature, as aleukaemic megakaryocytic myelosis. Its relationship to leukaemia remains uncertain; in some cases it appears to be a form of leukaemia or leucopenic leukaemia, but in other cases there is no evidence of leukaemia. Some cases, but not all, have a blood picture of a leuco-erythroblastic anaemia (Vaughan and Harrison, 1939). The condition is to be distinguished from Albers-Schönberg's disease, the latter, though it may produce a somewhat similar clinical picture, being primarily a disease of bone, with evidence of a familial incidence.

The marrow taken at biopsy from these four cases showed considerable variations, but there appeared to be certain distinguishing characteristics. The bone spicules appeared thicker than usual and a little increased in number. The marrow was patchy, certain areas consisted almost entirely of pale eosinophil, apparently fibrous, tissue, containing in its meshes a few scattered haemopoietic cells; other areas consisted of moderately hyperplastic haemopoietic marrow of the partly mature type with little or no fat. The haemopoietic cells were haemocyto blasts, primary erythroblasts, normoblasts, myelocytes, and polymorphonuclear leucocytes with a relative increase of immature forms; and the number of megakaryocytes usually appeared distinctly increased. Among the haemopoietic cells were fairly numerous oval and spindle-shaped cells, apparently fibroblasts, and an increase of pale

eosinophil, apparently fibrous, tissue. In certain instances the fibrous tissue appeared condensed with much closely packed deeply basophil material, and this was sometimes matted together, and had the characteristic appearance usually associated with the term myelosclerosis. The two cases in which this change was most obvious appeared from other evidence to be associated with leukaemia, and they are not included in the four cases described here. In summary, in the four cases of this type included in this section the marrow showed changes in varying degrees in the different cases: (1) a laying down of pale eosinophil, apparently fibrous, tissue, (2) an increase in the number of megakaryocytes, and (3) areas of dense basophil sclerotic tissue.

Two other cases were seen with anaemia, splenomegaly, and immature white cells in the peripheral blood, in which the marrow at autopsy showed the first two changes mentioned above, and could not, therefore, simply by the appearance of the biopsy sections, be distinguished from the cases described in this section. In both of them a post-mortem examination was made, and, although there was not more than slight enlargement of lymphatic glands macroscopically, the spleen, liver, and lymphatic glands on microscopic examination were thought to show the changes of Hodgkin's disease. Superficially even on microscopical examination there was considerable resemblance between the tissues in these two cases of Hodgkin's disease and those of the cases of myelosclerosis. The following points appeared to differentiate them. In the spleen and lymphatic glands of the cases diagnosed as Hodgkin's disease the normal architecture was practically destroyed, whereas in the cases of anaemia with sclerotic marrow the normal arrangement of lymph follicles and intervening pulp strands was preserved. In the cases of Hodgkin's disease there was some extramedullary haemopoiesis in the spleen and glands, but this was slight compared with the gross extramedullary haemopoiesis present in the other cases. Lastly, though it might be impossible to say whether an individual cell was a Hodgkin's giant cell or a megakaryocyte, the giant cells in the tissues in the case of myelosclerosis were usually large mononuclear cells with bizarre but single nuclei, whereas at least some of the giant cells in the tissues of the Hodgkin's disease cases were of the typical Hodgkin's type with multiple nuclei superimposed on one another at the centre of the cell.

In summary, the cases of refractory anaemia in this report have been divided according to the appearance of the bone marrow at biopsy, or at autopsy when no biopsy was performed, into four types. These four types with the recognized and unrecognized conditions with which they appear to be synonymous are as follows:

1. Refractory anaemia with partly mature cellular marrow, corresponding to the dysplastic phase of anaemia due to haemotoxic substances (Witts, 1936), or so-called pseudo-aplastic anaemia.
2. Refractory anaemia with hypocellular marrow, aplastic anaemia, or panmyelophthisis.



3. Refractory anaemia with immature cellular marrow, chronic granulocytopenia, probably including medullary pseudoleukaemia.

4. Refractory anaemia with fibrosis, sclerosis, and giant cell hyperplasia of the marrow, or myelosclerosis.

This classification is likely to be criticized on the grounds that such conditions as aplastic anaemia and myelosclerosis are well defined and distinct disease entities, and that the suggestion that they are related can cause only confusion. A careful consideration of the cases described here, however, makes it seem unlikely even on morphological grounds that these conditions are entirely distinct entities. It has already been pointed out that in almost all cases with biopsy and autopsy marrow available the autopsy marrow was less cellular than that taken at biopsy. Diminishing cellularity appears, therefore, to be a characteristic of the whole group. In one case (Case 5) the marrow was of the partly mature type and distinctly hypercellular at biopsy, and hypocellular at autopsy. In five cases the marrow was of the partly mature cellular type at biopsy and of the immature cellular type at autopsy. In one case with sclerotic marrow there were large areas of hypercellular marrow of the partly mature type, in another case with hypercellular partly mature marrow there were small areas of beginning fibrosis, while in several cases with hypercellular partly mature marrow the megakaryocytes were distinctly increased in number. In yet another case with hypercellular partly mature marrow the spleen *post mortem* showed numerous foci of extramedullary haemopoiesis containing megakaryocytes, a picture exactly like that found in cases with sclerotic marrow.

The changes in the liver, spleen, lymphatic glands, and other organs in the various types differed only in degree. Thus, though extramedullary haemopoiesis was greatest in the sclerotic type, it was present in some degree in all types. Haemosiderosis was greatest in cases with hyperplastic partly mature marrow, but was present in some degree in all types. Degeneration and necrosis of liver cells, probably too severe in many cases to be considered simply as secondary to the anaemia, was present in some cases of all of the first three types. There is, therefore, morphological evidence to suggest that these conditions are related, and there is a strong suggestion that refractory anaemia with marrow of the partly mature cellular type, seen much more often in biopsy than in autopsy specimens, is often a temporary phase in the course of other disorders of the haemopoietic system. There is, moreover, a growing volume of evidence that exposure to a single haemotoxin, benzol, may produce in human bone marrow each of the changes described here, with the exception perhaps of basophil sclerosis. It also seems likely that the number of cases of leukaemia following exposure to benzol that has now been reported is greater than can be explained on the grounds of coincidence (Mallory, Gall, and Brickley, 1939). These observations provide further evidence that the types of refractory anaemia described here are closely related conditions and should not be regarded as separate disease entities. The important question of the relationship of refractory anaemia to leukaemia

was deliberately omitted from this paper, as the evidence available is by no means conclusive. In the course of the investigation, however, a small number of cases was encountered, which suggested a close relationship between refractory anaemia and leukaemia. Several patients, in whose case there was marrow of the partly mature cellular type at biopsy and nothing in the clinical picture at that time to suggest leukaemia, subsequently died of leukaemia. In one case, where the bone marrow was confidently diagnosed at the time of biopsy as that of leucopenic myeloid leukaemia, the patient subsequently died and was found at autopsy to have hypoplastic marrow, with no evidence of leukaemia in any organ.

For purposes of clinical description the cases have been divided according to the type of marrow found to be present at biopsy, where this was done, and according to the type at autopsy, where no biopsy was done. In the following four sections an account is given of the morbid anatomy and clinical features of the four types. The average blood counts of the cases in the four groups at the time of admission are shown in the following table:

*Table showing the Average Blood Findings on Admission in 58 Cases of Refractory Anaemia, divided into Four Groups according to the Appearances of the Bone Marrow in Sections from Material obtained at Biopsy, or at Autopsy in the Few Cases in which no Biopsy had been performed.*

R.B.C. millions per c.mm.	Hb. % (Sahli)*	W.B.C. per c.mm.	Polymorphs		Platelets per c.mm.	M.C.V. cub. $\mu$	Reticu- locytes %
			%	per c.mm.			
<i>Partly mature cellular marrow (31 cases). (Average and limits)</i>							
1.8	40	3640	54	1964	169,000	96	2.9
3.5-0.6	81-13	8700-500	88-6	7395-55	580,000-10,000	120-77	12.5-0.0
<i>Hypoplastic marrow, aplastic anaemia (11 cases). (Average and limits)</i>							
1.7	42	2250	40	908	58,000	96	4.4
2.7-0.6	63-20	4600-1000	72-9	2387-99	166,000-12,000	107-75	8.0-1.0
<i>Immature cellular marrow, chronic granulocytopenia (12 cases). (Average and limits)</i>							
2.2	50	1850	20	375	79,000	102	1.8
2.9-0.9	70-21	4600-800	36-2	1656-60	148,000-32,000	118-94	3.3-0.4
<i>Sclerotic marrow, myelosclerosis (4 cases). (Average and limits)</i>							
1.15	27	2013	38	760	304,000	101	2.0
1.0-1.2	33-24	3100-1450	75-6	1088-120	308,000-300,000	106-94	3.7-0.4

\* Calibrated so that 100 per cent. of haemoglobin is equivalent to 13.8 gm. per 100 c.c. of blood.

### *3. Refractory Anaemia with Partly Mature Cellular Marrow, or Pseudo-aplastic Anaemia*

*Pathology.* Post-mortem examinations were made on six patients in whom marrow of this type was present at autopsy. The bodies in four cases were described as well nourished, in one as obese, and in one as that of a young man of exceptionally small and slight stature. The skin was pale in every case and there were no petechiae or ecchymoses present. In two cases, both

with other evidence of haemochromatosis, there was conspicuous light and dark brown mottled pigmentation of the skin, particularly of the hands and arms. In a third there was slight pigmentation of the same type and in a fourth freckling was perhaps abnormally conspicuous. There was slight atrophy of papillae at the edges of the tongue in two cases only. The subcutaneous fat was bright yellow in five of the six cases. Necrotic ulcers of both tonsils were present in one case; in the other five there was no sign of necrotic lesions or ulcerations. A few petechial haemorrhages were present in the pericardium of one case and in the epicardium and pleura of another.

There was no enlargement of the lymphatic glands in five cases; in one there was slight enlargement of the mesenteric and mediastinal glands. In this case and in two others the glands contained brown pigment visible to the naked eye. Microscopically in two cases there were very large amounts of haemosiderin situated in the pulp strands both inside and outside phagocytes. In two further cases there was moderate haemosiderosis, in one of them with some giant cells present in the pulp strands.

The average weight of the spleen was 160 gm., the largest weighing 300 and the smallest 110 gm. The capsule was free from adhesions and showed nothing remarkable. On the cut surface the pulp appeared abundant, with normal or inconspicuous markings. It was described as deep purple, greyish-purple, or maroon coloured in three cases; in the remaining three it was brown or brownish-purple from the presence of pigment. On microscopical examination the capsule and muscular trabeculae were usually increased in thickness. The lymph follicles were inconspicuous and the space occupied by the pulp appeared relatively increased. The pulp was very cellular, and contained in most instances very numerous erythrocytes, many lymphocytes, small numbers of polymorphonuclear leucocytes, and in five cases some primary erythroblasts and normoblasts. Myelocytes were occasionally present, and megakaryocytes were present in one case, but extramedullary erythropoiesis was much more prominent than leucopoiesis. Haemosiderin was present in the spleen in slight to moderate degree in five of the six cases.

The liver varied in weight from 1500 to 2160 gm. with an average weight of 1775 gm. It was described as light brown, golden brown, or mahogany brown in colour in different cases, and golden-brown pigment was visible in conspicuous amounts around the portal areas in two of the six cases. In four cases there was fatty degeneration or infiltration at the centres of the lobules. In one there was well-marked portal fibrosis of the multilobular type, and in another early fibrosis of the same type. There was slight or considerable haemosiderosis in five of the six cases. In the two cases with fibrosis of the liver the pancreas was deep brown in colour and on microscopical examination showed considerable haemosiderosis and an increase in fibrous interstitial tissue. Pigment was also present in the marrow in two cases, and in the choroid plexus and the basal layer of the epidermis in one.

The bone marrow in two cases appeared deep red in colour and cellular in all the bones examined, including the femur, reddish-grey or yellowish-grey,

containing considerable amounts of fat, in two, and uniformly yellow and apparently aplastic in two. The microscopical appearances of the bone marrow in this group have already been described. The appearances in autopsy sections differed little from those seen in biopsy sections. In four cases the autopsy sections were less cellular than those from biopsy and in two cases a relative increase in the proportion of immature cells present was noticed between the time of biopsy and autopsy. Haemosiderin was present in the marrow of two of the six cases.

In one patient there were signs of old healed tuberculosis in the upper lobe of the right lung; and in another an ectopic thymus the size of a lemon in the mediastinum. The immediate cause of death appeared to be bronchopneumonia in three cases, and a haemorrhage in the left motor area communicating with the subarachnoid space in another.

*Clinical Features.* Thirty patients had marrow of this type at biopsy. Seventeen were females and 13 were males. The average age at the time of onset of symptoms was 37 years, the oldest being 72 years and the youngest six months. Two patients stated that they had had sisters who had died of anaemia, the nature of which was not known, at the ages of 21 and 40 years respectively. The family histories in this group appeared otherwise unimportant.

In two cases the symptoms came on within a few months of the menopause; one patient, a man of 21 years at the time his symptoms appeared, had been a eunuch since an operation at the age of 16 for bilateral inguinal herniae and undescended testes. One patient had suffered from various kinds of bleeding from the age of five and had had his spleen removed at the age of 12. After this operation he had no bleeding for five years, but he remained severely anaemic and required 38 blood transfusions in the interval between the time of his splenectomy and his admission to the Rockefeller Hospital. The previous histories in the remaining cases seemed unimportant.

Of the 30 patients, 17 had been exposed to potentially toxic substances; five to benzol, one to a volatile organic insecticide, one to arsphenamine, and one to salvarsan; one was a luminous clock dial painter, who had pointed her brushes with her lips; two had taken atophan and one creosote, shortly before the onset of their symptoms; one had used a paraphenylene diamine hair dye and two others had dyed hair, but the nature of the dye used is not known; one had been exposed to rosin dust in unusual amounts and had a kind of addiction to the smell of this material; one gave a curious history of becoming sensitive to the smell of paint and paint removers and of having had to give up painting because these odours made her feel sick; and one lived over a garage and complained of the smell of fumes.

In five cases there was a history of a restricted diet. In the great majority the symptoms came on insidiously, and the exact date of onset was difficult to place. The presenting symptoms were commonly weakness, pallor, undue fatigue, dyspnoea, throbbing in the head, or palpitation. In two cases the condition started as an acute febrile illness, in one associated

with rigors, and in two the first symptoms were bleeding from the nose or the gums. On the whole these patients had few symptoms. Bleeding and purpura were noticed in a few cases, but were exceptional.

On examination the patients were usually described as well nourished. Most of them had slight irregular fever, though a small proportion remained afebrile for long periods at a time. Two had irregular bouts of fever separated by intervals of subnormal temperature, a Pel-Ebstein type of fever. In neither case was there anything else to suggest Hodgkin's disease, and in one this diagnosis was excluded by a post-mortem examination. Pallor was usually conspicuous and in a small minority of cases was associated with subicteric tint of the skin and conjunctivae.

Pigmentation of the skin varying from slight brown discoloration to conspicuous mottled dark pigmentation, chiefly on the hands, arms, and exposed parts, was present in five patients at the time they were first seen. In two of these there was other evidence of haemochromatosis *post mortem*, and a sixth patient developed considerable pigmentation and diabetes while under observation, and was found to have other evidence of haemochromatosis *post mortem*. Bleeding, purpura, and ecchymoses were seen in a few cases, but were exceptional; retinal haemorrhages were the commonest form and were observed at some time in 12 of the 30 patients.

There was no significant enlargement of lymphatic glands in any case. The spleen was just palpable at the time of admission in four cases. There was no definite enlargement of the liver in any case. Some atrophy of the papillae at the edges and tip of the tongue was present in five of the 30 cases. One patient of these five had also a rough reddened mucosa of the mouth and pharynx, and had had some difficulty in swallowing. Another patient had small ulcers of the mucous membrane of the mouth and the upper lip. Two had sore gums, one with small areas of necrosis, and one had leucoplakia of the nasal septum and buccal mucosa. In one case there was an asymmetrical diminution of vibration sense in the legs; no other neurological abnormalities were detected.

*Laboratory investigations.* The average blood counts in 31 patients at the time of admission were: red cells 1,800,000 per c.mm., haemoglobin 40 per cent., white cells 3640 per c.mm., polymorphonuclear leucocytes 54 per cent., platelets 169,000 per c.mm., mean corpuscular volume 96 cub.  $\mu$ , and reticulocytes 2.9 per cent. In stained films the red cells were well coloured, there was usually slight or moderate macrocytosis and anisocytosis, and poikilocytosis was seen quite frequently, but was usually not severe. Myelocytes, and less frequently normoblasts, were present in small numbers in the films of about half the cases seen.

Free hydrochloric acid was present in the gastric juice in 21 of the 22 cases examined, but usually only after the administration of alcohol or histamine. The icterus index determined in 14 cases was above normal at some time (values between 6 and 20) in eight. Haemolysis of red cells in saline in 10 cases began in an average concentration of 0.44 per cent. and was

complete at 0.33 per cent. (limits 0.5 to 0.26 per cent.); the average values in 17 control observations in normal subjects were 0.44 to 0.33 (limits 0.46 to 0.28). The fragility of the red cells in saline was therefore practically normal in these six cases.

*Clinical course.* The average duration of the illness in the 19 patients who have died was 26 months, the longest  $9\frac{1}{2}$  years and the shortest three months. Of the 11 patients who are still alive, five have had complete remissions. Of these five patients four had been exposed to benzol and one to arsphenamine. Of the remaining six patients still alive one has had three temporary remissions in five years (Case 22), one is alive  $5\frac{1}{2}$  years after her first symptoms, but it is not known whether she is anaemic at the time of writing, and four are alive but severely anaemic, 23 months, 27 months, four years, and  $7\frac{1}{2}$  years respectively after their first symptoms. The course of this type of refractory anaemia is therefore variable, but on the whole more benign than that in the other three types, and spontaneous remissions either partial or complete are not uncommon.

Bleeding and necrotic ulcerations occurred, but were relatively uncommon. Purpura or ecchymoses were seen in nine of the 31 patients at some time in their illness, and retinal haemorrhages were seen in 12. Bleeding from the nose or gums occurred in 12, but except as a terminal event was neither as profuse nor as stubborn as in the group with hypocellular marrow. Superficial necrotic ulcers of the tonsils occurred in two patients, both of whom subsequently had complete remissions. In one patient with marrow of the partly mature cellular type at biopsy and autopsy, there was terminally an extensive slough of the tonsils and the posterior portion of the tongue. In two patients with marrow of the partly mature cellular type at biopsy and of the immature cellular type at autopsy, there occurred terminally sloughs of the gums and jaw in one and of the ischio-rectal fossa in the other, and in one patient with marrow of the partly mature type at biopsy and severely hypoplastic marrow at autopsy there was necrotic ulceration of the gums appearing shortly before death.

Fever, usually slight in degree, was present in all but two of these patients at some time in their illness; in those patients in whom the condition took a benign or prolonged course fever was slight or absent for long periods. In a few cases there were occasional rigors. The spleen, which was just palpable at the time of admission in four patients, became palpable at some time in the course of the illness in four more. It was, however, not felt more than a few centimetres below the costal margin, except in one case, the patient with three temporary remissions in five years, in whom it was enlarged almost to the umbilicus at times when the patient was severely anaemic, but diminished in size and became barely palpable during remissions. In one other patient (Case 26) the spleen varied significantly in size, enlarging periodically in association with rise of temperature, falling blood count, and increase of the icterus index.

Pigmentation of the skin as already described was present in five patients

when they were first seen, and appeared in a sixth while he was under observation. This last patient also developed terminally severe diabetes.

The immediate cause of death most commonly was bronchopneumonia, two patients died shortly after cerebral haemorrhage, one died with an acute mastoiditis, and one with diabetes complicated by an abscess over the mastoid. In several there was no apparent cause other than anaemia, and in these cases the approach of death was indicated by diminishing benefit from transfusions, by the occurrence of slight jaundice, and by increasing and sometimes uncontrollable haemorrhage from the nose and gums.

### *Case Reports*

Refractory anaemia with partly mature cellular marrow, possibly associated with the prolonged use of a paraphenylene diamine hair dye. Prolonged course with few symptoms.

*Case 1.* A married Italian woman who first complained of weakness at the age of 48 years, ten months before the time of her first admission to the Rockefeller Institute Hospital. Her family history and her previous history appeared unimportant, except that the menopause had occurred five months before the onset of her symptoms. For ten years she had had her hair dyed about six times a year with a paraphenylene diamine hair dye; six months before the onset of her symptoms she also had a permanent wave (cf. Baldrige, 1935). Her diet had been a moderately restricted one, but appeared qualitatively adequate. Two months before admission she was found to be anaemic with 1,400,000 red cells per c.mm., haemoglobin 28 per cent., and 3,600 white cells per c.mm. She was treated with iron and with liver extract by mouth and by injection.

*Physical examination.* Temperature 98° F., pulse 72. Well developed and wellnourished. Strikingly pale, with no jaundice. Tongue normal. No haemorrhages. Chest normal. Haemic murmur. No enlargement of liver, spleen, or lymphatic glands. No neurological abnormality.

*Laboratory investigations.* Blood count on admission: red cells 1,100,000 per c.mm., haemoglobin 32 per cent., colour index 1.4, mean corpuscular volume 120 cub.  $\mu$ , reticulocytes 1.2 per cent., platelets 240,000 per c.mm., white cells 2,400 per c.mm.; differential count, polymorphonuclears 52 per cent., lymphocytes 39 per cent., eosinophils 8 per cent., myelocytes 1 per cent. One normoblast was seen in counting 100 white cells. Icterus index 6. Test meal: free hydrochloric acid present. Barium meal: no abnormality. Radiographs of chest showed no abnormality; those of long bones showed slight thickening of periosteum only. Average daily urobilinogen excretion, urine 0.4 mg., stools 32.0 mg.

*Biopsy of sternal bone marrow.* Section shows quite densely hypercellular marrow without fat. There are fairly numerous haemocytoblasts often in small groups, numerous primary erythroblasts in various stages, and some normoblasts; relatively few myelocytes, often eosinophil, and polymorphonuclear leucocytes. Numerous megakaryocytes. The marrow is therefore a partly mature, hypercellular, and predominantly erythropoietic one.

*Further course.* This patient was under observation for three years, and was admitted to hospital on eight occasions. Throughout this time she remained afebrile except for occasional small rises of temperature to 99° or 100° F. Her blood count remained in the neighbourhood of 1,000,000 red cells per c.mm. and 30 per cent. of haemoglobin; there were usually a few

myelocytes in blood films. On one occasion 12 per cent. of myelocytes were seen. In spite of this count the patient had remarkably few symptoms. There were occasional small ulcers in the mouth, and on one occasion a single retinal haemorrhage was seen. The patient was able to be at home and up and about for considerable periods with a red cell count below 1,000,000 per c.mm. One year after her first admission, her red cells being 800,000 per c.mm. and haemoglobin 21 per cent., she was given a transfusion. During the next two years her condition changed very little, and her weight increased. Except after transfusions, of which she was given eight in all, her red count and haemoglobin remained at about the same level as before. Her white cells were usually between 3,000 and 4,000 per c.mm., with about 30 per cent. of polymorphonuclears. No further myelocytes were seen in films, but eosinophils were usually increased, and in one count were as high as 20 per cent. There were numerous platelets in stained films, and recently showers of very large platelets have been seen in some films. The patient's condition at the time of writing remains almost unchanged, four years after her first symptoms.

Refractory anaemia with partly mature cellular marrow in a child. Prolonged course with few symptoms.

*Case 2.* A Norwegian female child who was first known to be anaemic at the age of nine months. She was the second child of healthy parents, was born at term weighing  $5\frac{1}{2}$  lb., and was breast fed for five months. At about six months of age she appeared very pale. When nine months old she was admitted to the Norwegian Hospital where she remained till she was admitted to the Rockefeller Institute Hospital at the age of three years. She had been treated with liver extract injections, iron and ammonium citrate in large doses, and about 28 blood transfusions. None of these measures had produced more than temporary benefit.

*Physical examination.* Temperature  $99.5^{\circ}$  F., pulse 100. A well developed and well nourished child, alert and apparently of normal mental development. Tongue normal. No haemorrhages. Chest and heart normal. No enlargement of liver, spleen, or lymphatic glands. No neurological abnormality.

*Laboratory investigations.* Blood count on admission: red cells 3,500,000 per c.mm., haemoglobin 71 per cent.; colour index 1.0, mean corpuscular volume 88 cub.  $\mu$ , platelets 120,000 and 290,000 per c.mm. on different occasions, white cells 7,650 per c.mm.; differential count, polymorphonuclear leucocytes 45 per cent., eosinophils 4 per cent., lymphocytes 50 per cent., monocytes 1 per cent.

*Biopsy of sternal marrow.* Hypercellular marrow separated by areas of haemorrhage (Giemsa stain). The cellular areas show few haemocytoblasts, numerous myelocytes, and fairly numerous polymorphonuclear leucocytes; relatively fewer primary erythroblasts and few normoblasts. Numerous megakaryocytes. A partly mature cellular and predominantly leucopoietic marrow.

*Further course.* The patient was admitted on 15 occasions in the next  $3\frac{1}{2}$  years. She usually had mild irregular fever to  $100^{\circ}$  or  $101^{\circ}$  F. She had no symptoms other than those due to her low blood count, and no haemorrhages. Except when her count was very low, she was active and had a good appetite. She was treated at different times with raw liver, ventriculin, and Vegex by mouth, and with Congo Red by injection. She was also given 29 transfusions, usually of 350 c.c. each. Her haemoglobin was as high as 60 per cent. after transfusions, and on one occasion was as low as 9 per cent. with a red cell count of 500,000 per c.mm. On discharge her blood count



was, red cells 800,000 per c.mm., haemoglobin 15 per cent., platelets 290,000 per c.mm., white cells 4,650 per c.mm.; differential count, polymorphonuclears 58 per cent., basophils 2 per cent., eosinophils 10 per cent., lymphocytes 26 per cent., monocytes 4 per cent. A year later, and 6½ years after her first symptoms, when the patient was last heard of, her condition had changed little and she was continuing to receive transfusions. She was then seven years old and was known to have received 60 transfusions.

Refractory anaemia with partly mature cellular marrow, with development of mild haemochromatosis.

*Case 3.* A lawyer of Austrian extraction who first complained of pallor and weakness at the age of 51 years, 18 months before his admission to the Rockefeller Institute Hospital. His previous and family history appeared unimportant and his diet had been good. He lived over a garage and had often complained of the smell of fumes. Shortly after the onset of his symptoms a blood count was as follows, red cells 1,200,000 per c.mm., haemoglobin 30 per cent., colour index 1.25. A diagnosis of pernicious anaemia was made, and on treatment with liver extract by injection the haemoglobin increased to 65 per cent. The patient then went six months without treatment. Four months before admission he was again severely anaemic, and on this occasion intensive treatment with liver extract was without effect. Bleeding occurred from the gums. The patient had three transfusions and a prolonged course of liver therapy with no permanent benefit.

*Physical examination.* Temperature 99.0° F., pulse 108. Well developed and well nourished. Subicteric pallor. Tongue normal. No retinal or other haemorrhages. Chest normal. Haemic murmur. Blood pressure 112/70. No enlargement of spleen or lymphatic glands. Liver just palpable. No neurological abnormality.

*Laboratory investigations.* Blood count on admission, red cells 1,400,000 per c.mm., haemoglobin 30 per cent., colour index 1.07, mean corpuscular volume 90 cub.  $\mu$ , reticulocytes 1.4 per cent., platelets 68,000 per c.mm., white cells 1,150 per c.mm.; differential count, polymorphonuclear leucocytes 36 per cent., lymphocytes 58 per cent., monocytes 2 per cent., eosinophils 3 per cent., basophils 1 per cent. Test meal, free hydrochloric acid present.

*Sternal marrow biopsy.* Slightly hypocellular marrow with large pink structureless areas and islands of apparently very active haemopoiesis. These areas show groups of haemocyto blasts, primary erythroblasts, and normoblasts, with relatively fewer myelocytes and very few polymorphonuclear leucocytes. No megakaryocytes seen.

*Further course.* The patient was discharged after two months and then attended as an out-patient, with periodic admissions for transfusions. He was usually afebrile, but occasionally had slight fever. Transfusions, of which he had 13, had less and less effect, and the blood count remained near 1,000,000 red cells per c.mm. Finally, bleeding from the gums reappeared, with purpura on the extremities, the condition failed to respond to transfusion, and the patient died one year after admission and 2½ years after his first symptoms. His last blood count was, red cells 1,100,000 per c.mm., haemoglobin 23 per cent., colour index 1.07, mean corpuscular volume 86 cub.  $\mu$ , white cells 1,900 per c.mm.; differential count, polymorphonuclear leucocytes 9 per cent., lymphocytes 66 per cent., monocytes 13 per cent., eosinophils 12 per cent.

*Summary of autopsy findings.* Well developed and well nourished. Pale, with multiple irregular areas of brownish-black pigmentation on back of

hands and on arms. Bright yellow subcutaneous fat. Lungs, bronchopneumonia in lower lobes. Heart not abnormal. Spleen (160 gm.) with inconspicuous markings and deep-brown colour on cut surface. Liver (1,500 gm.) with firm golden-brown cut surface and light areas around central veins. Kidneys not abnormal. Pancreas deep brown in colour with normal pattern on cut surface. No enlargement of lymphatic glands. Mesenteric glands deep brown on cut surface. Sternal, costal, and vertebral marrow reddish-yellow, and appears to contain considerable quantities of fat; femoral marrow fatty with irregular apparently cellular areas.

*Summary of microscopical findings.* Marrow, femoral section fatty with few small islands of cellular marrow; remaining sections vary in cellularity, but on the average the cellularity is about normal. There are small groups of haemocytoblasts, numerous primary erythroblasts, and few normoblasts; some myelocytes, but very few polymorphonuclear leucocytes. No megakaryocytes seen. The marrow is therefore immature, with erythropoiesis more active than leucopoiesis. Liver, considerable haemosiderosis and early multilobular fibrosis. Pancreas, haemosiderosis and some increase of fibrous tissue. Lymphatic glands, haemosiderosis in pulp strands in and outside of phagocytes; increased number of plasma cells. Lungs, bronchopneumonia.

Refractory anaemia with hypercellular partly mature marrow at biopsy and marrow of normal or slightly reduced cellularity at autopsy; possibly associated with taking of creosote. Splenectomy.

*Case 4.* A married American woman who first complained of dyspnoea at the age of 39 years, six months before the time of her admission to the Rockefeller Institute Hospital. Her family history appeared unimportant. She had been married 16 years, but had had no pregnancies. Nine years previously a right ovarian cyst had been removed. Menstruation was regular, but scanty in amount. Her diet had been good. Eight months before admission the patient was in China and, being troubled with what she described as bronchitis, took a creosote preparation over a period of two or three weeks. Six weeks later she complained of dyspnoea and was noticed to be pale. She set out for Europe, and during the voyage had severe bleeding from the gums and petechiae on the arms and legs. On arrival in London she was too weak to walk and entered a nursing home. She was found to have a red cell count of 700,000 per c.mm., and was treated with three transfusions and ventriculin. After two months she returned to New York. In spite of a further transfusion and intensive treatment with parenteral liver extract and ventriculin, she continued to suffer from weakness, dyspnoea, and bleeding from the nose, gums, and vagina.

*Physical examination.* Temperature 99.6° F., pulse 104. Well developed and well nourished, but appears pale and ill. Small retinal haemorrhages, and petechial haemorrhages on soft palate. Tongue normal, gums bleeding. Haemic cardiac murmur. No enlargement of liver, spleen, or lymphatic glands. No neurological abnormality.

*Laboratory investigations.* Blood count on admission, red cells 600,000 per c.mm., haemoglobin 13 per cent., colour index 1.08, mean corpuscular volume 84 cub.  $\mu$ , reticulocytes 2.4 per cent., platelets 42,000 per c.mm., white cells 4,200 per c.mm.; differential count, polymorphonuclear leucocytes 24 per cent., lymphocytes 72 per cent., monocytes 3 per cent., eosinophils 1 per cent. Test meal, free hydrochloric acid present after alcohol. Haemolysis of red cells in saline normal.

*Biopsy of sternal marrow.* Section shows quite densely hypercellular

marrow with no fat. Under low power there are conspicuous groups of haemocyto blasts, erythro blasts, and normo blasts. Under high power these same groups are seen and, in addition, a few myelocytes, often eosinophil, and a few polymorphonuclear leucocytes. No megakaryocytes seen. The marrow is hypercellular, partly mature, and predominantly erythropoietic.

*Further course.* This patient had slight irregular fever to 100° or 101° F. throughout the period she was under observation. She was given a course of intravenous liver extract, but her condition deteriorated. Intravenous ascorbic acid failed to check the bleeding from her gums. She was given six transfusions in a period of 3½ months, but improvement after transfusions became progressively less, until in the latter part of the period the blood count returned to its previous level almost immediately after each transfusion. As this observation suggested that the patient was haemolysing the blood given her by transfusion, and as she was thought to have a cellular marrow, it was decided as a last resort to remove her spleen. She was therefore transferred to the New York Hospital, where splenectomy was performed by Dr. Andrus under nitrous oxide, ethylene, and open ether anaesthesia, without undue difficulty. The patient was given a transfusion pre-operatively and during the operation. Apart from some bleeding from the gums and retinal haemorrhages the patient's post-operative course was uneventful up to the time, six days after operation, that she was returned to the Rockefeller Institute Hospital. One week after operation she developed numerous petechiae and ecchymoses on the skin, bleeding from the lips, gums, nose, and mouth, and a small necrotic ulcer on the inside of the lower lip. She was given a further transfusion, but died four days later, four months after her first admission and 11 months after her first symptom.

*Summary of post-mortem findings.* Well developed and well nourished. Recent almost unhealed splenectomy scar. Multiple subcutaneous ecchymoses. Ulceration of oral mucous membrane; mouth full of old blood. Considerable amounts of soft blood clot at site of splenectomy in left upper abdomen. Right pleural cavity obliterated by fibrous adhesions. Subpericardial haemorrhages. Congestion and oedema of lower lobes of lungs. Liver (920 gm.) uniformly yellow-brown in colour, with small depressions around central veins on cut surface. Haemorrhagic cyst 3 cm. in diameter in right ovary. Sternal, costal, vertebral, and femoral marrow appears yellow and fatty, except for area in sternum at line of third interspace, which is greyish red and appears cellular.

*Summary of microscopical findings.* Marrow, section of femoral marrow completely fatty, that of vertebral and sternal marrow has normal architecture and is about normal in degree of cellularity. There are few haemocyto blasts, numerous primary erythro blasts in various stages, and a few normo blasts. Very few myelocytes, often eosinophil, and few polymorphonuclear leucocytes. No megakaryocytes seen. Liver, moderate haemosiderosis around portal areas. Two sections of lymphatic glands show normal architecture, with lymph follicles and very conspicuous pulp strands. Under high power there is slight extramedullary haemopoiesis, chiefly erythropoiesis of same type as in marrow, with fairly numerous primary erythro blasts and a very few myelocytes. No abnormality seen in kidney, suprarenal, lungs, or pancreas.

Refractory anaemia with change in type of marrow from slightly hypercellular and partly mature to hypocellular.

*Case 5.* An American printer, 59 years old, was first noticed to be pale six

months before admission to the hospital. His family history seemed unimportant and there was no history of exposure to toxic substances. Five years previously a papilloma of the bladder had been treated successfully by diathermy. In the six months before he was admitted his haemoglobin varied between 25 and 40 per cent. He was treated with liver extract and iron without effect, and received one transfusion. He was noticed to be developing a brownish pigmentation of the skin.

*Physical examination.* Temperature 99° F., pulse 100. Well nourished. Moderate pallor with slight bronze pigmentation scattered over the body, but more marked on the face, neck, arms, and hands. No retinal or other haemorrhages. Tongue and throat normal. Chest normal. Haemic cardiac murmur. Blood-pressure 110/70. No enlargement of liver, spleen, or lymphatic glands. No neurological abnormality.

*Laboratory investigations.* Blood count on admission, red cells 1,400,000 per c.mm., haemoglobin 38 per cent., colour index 1.4, mean corpuscular volume 118 cub.  $\mu$ , reticulocytes 5.9 per cent., white cells 5,000 per c.mm.; differential count, polymorphonuclear leucocytes 44 per cent., lymphocytes 23 per cent., monocytes 25 per cent., eosinophils 5 per cent., myelocytes 3 per cent. Icterus index 3. Saline fragility, haemolysis begins at 0.44 per cent. and is complete at 0.34 per cent. (control 0.44 to 0.34 per cent.). Test meal, free hydrochloric acid present after alcohol and histamine. Twenty-four hour urobilinogen output (average of three 3-day periods), urine 1 mg., stools 83 mg. Stools, no occult blood.

*Sternal marrow biopsy.* Bone spicules and architecture normal. Marrow slightly hypercellular, differing from normal only in showing a relative increase of immature forms and decrease of normoblasts and polymorphonuclears. More leucopoietic than erythropoietic cells. Megakaryocytes appear almost normal.

*Further course.* The patient remained well enough to be up and about almost up to the time of his death 17 months later. He received 11 transfusions and various other treatments with no evidence of any permanent benefit. He remained afebrile and no bleeding occurred. A second sternal biopsy 11 months after the first showed a considerable decrease in cellularity, and a relative increase in erythropoietic and decrease in leucopoietic cells. Anaemia and leucopenia persisted, no abnormal cells were seen in the blood films, and shortly before death his blood count was, red cells 600,000 per c.mm., haemoglobin 11 per cent., white cells 2,950 per c.mm. He died two years after the onset of his first symptoms.

*Summary of autopsy findings.* Well developed and well nourished. Slight bronze pigmentation of skin, especially of arms and wrists. Sclerae slightly icteric; subcutaneous fat bright yellow. Heart (570 gm.) considerably enlarged. Lungs, healed tuberculosis in right upper lobe. Spleen (120 gm.) with normal pattern and golden-brown pigmentation. Liver (1,570 gm.) with golden-brown pigmentation, especially of portal areas. Scar in bladder. No enlargement of lymphatic glands. Costal, sternal, vertebral, and femoral marrow appears to consist uniformly of yellow fat.

*Summary of microscopical findings.* Marrow moderately hypocellular with strands and groups of very cellular haemopoietic marrow between fat cells, containing haemocyctoblasts, erythroblasts, and normoblasts, with relatively few myelocytes and very few polymorphonuclears. No megakaryocytes seen. Liver, central fatty degeneration and very considerable haemosiderosis. Spleen congested, with moderate haemosiderosis, slight erythropoiesis, and occasional myelocytes. Lymphatic gland (mediastinal), large area of hyaline

fibrous tissue; haemosiderosis. Oedema and congestion of lungs, with fibrinopurulent pleurisy.

Refractory anaemia with two-years course, and change in type of marrow from partly mature cellular to immature cellular.

*Case 6.* An Italian consulting engineer, 53 years old, who had lived in America for 20 years, was first seen to be pale one year before the time of his admission to the Rockefeller Hospital. He had made no complaint, but his haemoglobin was found to be 29 per cent. His family history was unimportant. His only previous illnesses were malaria at the age of 10 and typhoid fever at 21 years. He was treated with liver extract, and his haemoglobin increased to 45 per cent. Cystic forms of *Giardia lamblia* were found in the duodenal contents and stools, and for this he was given colonic irrigations and duodenal lavage, including one lavage with salvarsan, the last being followed by fever and a rigor. Three or four months before admission he developed a painful ischiorectal abscess which discharged pus and continued to drain up to the time of admission.

*Physical examination.* Temperature 99.2° F., pulse 96. Well developed and well nourished, but pale and ill. Tongue and throat normal. Chest normal. Heart slightly enlarged with systolic and diastolic murmurs at mitral area. Blood pressure 110/60. Liver palpable 6.5 cm. below costal margin; spleen just palpable. No enlarged lymphatic glands. No neurological abnormality.

*Laboratory investigations.* Blood count on admission, red cells 1,100,000 per c.mm., haemoglobin 24 per cent., colour index 1.14, mean corpuscular volume 105 cub.  $\mu$ , reticulocytes 1.6 per cent., platelets 518,000 per c.mm., white cells 4,250 per c.mm.; differential count, polymorphonuclear leucocytes 48 per cent., lymphocytes 41 per cent., monocytes 1 per cent., eosinophils 10 per cent. Test meal, free acid present after histamine. Stools, *Amoebae coli* and cysts of *Giardia lamblia*; no occult blood.

*Biopsy of sternal marrow.* Normal bone spicules. Hypercellular marrow with little fat in most areas. Some sections show solid sheets of cells. These are mostly leucopoietic, with very numerous polymorphonuclear leucocytes and myelocytes. Groups of early erythroblasts and a few normoblasts. Very numerous megakaryocytes of atypical appearance.

*Further course.* The patient had slight fever. As time went by transfusions became necessary at increasingly frequent intervals. Finally, he developed dyspnoea with an enlarged heart, rapid pulse, increasing enlargement of the liver, ascites, and pitting oedema of the legs. He died 13 months after his first admission and two years after his first symptoms. His last blood count was red cells 1,100,000 per c.mm., haemoglobin 19 per cent., white cells 3,950 per c.mm.; differential count, polymorphonuclear leucocytes 48 per cent., lymphocytes 38 per cent., eosinophils 10 per cent., basophils 4 per cent. No abnormal cells were seen in differential counts.

*Summary of autopsy findings.* Obesity, with oedema of feet, ankles, and sacrum. Yellowish subcutaneous fat. Oedema and congestion of lungs. Heart enlarged, with left ventricular hypertrophy; heart valves normal except for thickening and some calcification of aortic cusps, without narrowing of orifice. Liver (2,795 gm.), brownish-red and firm with normal markings. Spleen (375 gm.), greyish-purple with greyish-yellow cut surface showing splenic markings less conspicuous than usual. Kidneys not abnormal. Pancreas rusty yellow. Femoral, vertebral, costal, and sternal marrow greyish-red and cellular.

*Summary of microscopical findings.* Marrow, with normal bone spicules, hypercellular, but noticeably less so than in biopsy section, with normal architecture in most areas. The great majority of cells are primary erythroblasts, with some myelocytes and scattered normoblasts. The marrow was therefore of the immature cellular type. Megakaryocytes in about normal numbers. Liver, severe central necrosis with considerable haemosiderosis; occasional lymphocytes, but no leukaemic infiltration. Spleen, normal germ centres, and outside them large numbers of erythroblasts and lymphocyte-like cells. Some haemosiderosis. Kidneys normal. Lymphatic glands, large amounts of haemosiderin marking out pulp strands.

Refractory anaemia with change in type of marrow from partly mature cellular to immature cellular.

*Case 7.* A married American housewife, aged 66 years, first complained of weakness about seven months before her admission to the Rockefeller Institute Hospital. She had had a partial thyroidectomy at the age of 58 years for toxic goitre. One year previously she had had an operation for urethral caruncle. She had always eaten little meat and for two years practically none. Five months before admission she complained of frequency and dysuria. Her blood count then was, red cells 730,000 per c.mm., haemoglobin 20 per cent., white cells 6,500 per c.mm.; differential count, polymorphonuclear leucocytes 63 per cent., lymphocytes 37 per cent. A sternal marrow biopsy showed a partly mature hypercellular marrow, with apparently normal white cell maturation and almost complete absence of red cell precursors; megakaryocytes in normal or slightly increased numbers. Free hydrochloric acid was present in the stomach, and radiographs of the bones and intestinal tract showed no abnormality. She was treated with liver extract and iron without any response, and subsequently with transfusions.

*Physical examination.* Temperature 99.0° F., pulse 88. Well developed and well nourished, with moderate pallor. No retinal or other haemorrhages. Tongue and throat normal. Chest normal. Soft haemic cardiac murmur. Moderate arteriosclerosis. Blood pressure 135/60. No enlargement of liver, spleen, or lymphatic glands. No neurological abnormality.

*Laboratory investigations.* Blood count on admission (shortly after four transfusions), red cells 2,100,000 per c.mm., haemoglobin 50 per cent., colour index 1.2, mean corpuscular volume 101 cub.  $\mu$ , reticulocytes 0.4 per cent., platelets 290,000 per c.mm., white cells 3,550 per c.mm.; differential count, polymorphonuclear leucocytes 68 per cent., lymphocytes 26 per cent., monocytes 4 per cent., eosinophils 1 per cent., basophils 1 per cent. Red cell fragility, haemolysis begins at 0.44 per cent., and is complete at 0.36 per cent. saline (control 0.44 to 0.34 per cent). Twenty-four hour stool urobilinogen (average of three 3-day periods) 84 mg. Urine, few leucocytes. Pyelograms, no evidence of hydronephrosis.

*Further course.* For several months this patient had irregular fever rising at times to 104° F., with occasional rigors. Thereafter she had only slight fever. She was given 15 transfusions in 15 months, and obtained less and less benefit from them. No abnormal cells were seen in differential counts, and there was no enlargement of the liver, spleen, or lymphatic glands. She died 15 months after admission and 21 months after her first symptom. Blood count shortly before death, red cells 600,000 per c.mm., haemoglobin 10 per cent., white cells 5,200 per c.mm.

*Summary of autopsy findings.* Well nourished. Slight oedema of ankles. Subcutaneous fat noticeably yellow. Slight enlargement of heart. Lungs

normal. Spleen (140 gm.), firm with normal colour and markings. Liver (1,420 gm.), deep golden-brown, with pigment around portal areas. No enlargement of lymphatic glands. Sternal, costal, vertebral, and femoral marrow appear pale-yellow and fatty.

*Summary of microscopical findings.* Marrow diffusely but not densely cellular. Some sections show no fat, others contain less than a normal amount of fat cells with normal architecture. The cells are almost entirely immature basophil ones, with occasional eosinophil myelocytes and small scattered groups of erythroblasts. No normoblasts or polymorphonuclear leucocytes. Spleen, some haemosiderosis. Liver, severe central necrosis and fatty infiltration, and very conspicuous haemosiderosis. Lymphatic gland appears normal.

#### 4. *Refractory Anaemia with Hypercellular Marrow, or Aplastic Anaemia.*

*Pathology.* Of 11 patients with marrow of this type at biopsy, four were examined *post mortem*. Two patients with marrow of the partly mature cellular type at biopsy had hypocellular marrow *post mortem*. Post-mortem observations were made, therefore, on six patients with hypocellular marrow. The bodies were described as well nourished in three of these cases, obese in two, and emaciated in one. There was conspicuous pallor in all, and in some cases a slightly yellow tint of the skin and conjunctivae. Unusual yellowness of the subcutaneous fat was noted in four of the six cases. Pigmentation of the skin was not seen; the nails were normal, and there was slight atrophy of papillae at the edges of the tongue in one case only. External haemorrhages varied from petechiae to large subcutaneous ecchymoses, and were present in three of the six cases. There were small ulcers of the lips, tongue, buccal mucosa, and terminal ileum in one case, necrotic ulceration of the right tonsillar fossa in one, a large necrotic ulcer of the upper gums and palate in a third, and a necrotic ulcer of the perineum in a fourth. As lesions of this kind were most commonly seen in the group with immature cellular marrow, they will be described more fully in the next section.

Internal haemorrhages were found in different cases in the gastro-intestinal tract, the pericardium, the epicardium, the peripelvic tissue of the kidney, the subdural space, and the uterus and vagina.

The lymphatic glands appeared normal in size and appearance in every case. Microscopically in several cases the glands appeared somewhat depleted of cells, in two cases there was moderate haemosiderosis, and in one there was slight extramedullary haemopoiesis, indicated by the presence of occasional myelocytes and normoblasts.

The spleen varied in weight from 110 to 200 gm., with an average weight of 141 gm. This was the smallest average weight of the four groups, and would have been smaller but for a patient with a spleen weighing 200 gm., in whom there was evidence of old coronary infarction, oedema of the legs, and other signs of heart failure. The capsule of the spleen was free from adhesions and showed nothing remarkable. The consistency was soft, and the cut surface showed little pulp and normal or rather prominent markings.

Some golden-brown pigment was visible to the naked eye in two cases. Microscopically the pulp usually contained fewer cells than normal and sometimes conspicuously few red cells; there was slight to moderate haemosiderosis in five of the six cases, and a few myelocytes, primary erythroblasts, and normoblasts were also present in five of the six.

The liver varied in weight from 710 to 1,890 gm., with an average weight of 1,198 gm. The external surface was usually smooth and pale yellowish-brown in colour. The cut surface was similar in colour, and its appearance suggested the presence of fatty infiltration or degeneration; in one case it was described as golden-brown, and in one the pattern was accentuated by the presence of yellow areas around the central veins. On microscopical examination there were varying degrees of fatty degeneration and infiltration; in one case there was advanced necrosis at the centres of the lobules, and in two there was slight haemosiderosis.

The bone marrow examined in the sternum, a rib, a vertebra, and a femur was described as completely fatty, fatty with scattered red areas, or mottled reddish-yellow in all the bones examined. The microscopical appearances have already been described; those seen *post mortem* did not differ qualitatively in any way from those seen in biopsy sections. Slight haemosiderosis was present in the marrow of two cases.

In the remaining organs the changes were those seen in any severe anaemia. In one case enlargement of the pineal gland (to  $0.4 \times 0.4 \times 1.0$  cm.) was present.

The immediate cause of death appeared most frequently to be a respiratory complication, bronchopneumonia in three cases, associated with a lung abscess in one, and severe pulmonary oedema in two. In one case death was attributed to a subdural haemorrhage.

*Clinical features.* Eleven patients had marrow of this type at biopsy; five were female and six were male. The average age at the time of onset of the symptoms was 39 years, the youngest being 18 and the oldest 64 years. The family histories seemed unimportant, as did the previous illnesses. One patient, a woman of 59 years, gave a history that she had bruised unusually easily since the time of the menopause, and in another the symptoms had begun four months after the menopause. One, a man of 43 years, was a eunuch. There was a history of a restricted diet in two cases. Four of the 11 patients had been exposed to benzol, one to photographic chemicals, including hydroquinone, one had used a paraphenylene diamine hair dye, and one had been treated for tonsillitis with a proprietary preparation containing phenacetin.

The patients were usually severely anaemic when they first noticed symptoms or when some minor ailment led to the discovery of their anaemia. The presenting symptoms were weakness, increasing fatigue, dyspnoea on exertion, pallor, spontaneous bruising, or bleeding from the nose or gums. Bleeding occurred at some period of the illness in seven of the 11 cases. The patients were usually well nourished and conspicuously pale. Their



complexion was sallow, or exceptionally there was slight lemon-yellow jaundice. Tachycardia and slight irregular fever or fever swinging to  $103^{\circ}$  or  $104^{\circ}$  F. was present at some time in all but the mildest cases. Purpura or less commonly large ecchymoses occurred in seven cases and retinal haemorrhages were present in eight. These varied from single small haemorrhages to multiple large flame-shaped ones, and in one case there was a subhyaloid haemorrhage. In a patient in whom a remission of his anaemia after multiple retinal haemorrhages and secondary glaucoma had occurred, there was severe and permanent impairment of vision. The tongue and nails appeared normal except for slight atrophy of papillae at the edges of the tongue in one patient, a man of 54 years. There was no enlargement of lymphatic glands, except of the cervical glands in a patient with an infected throat. Systolic murmurs, presumably haemic in nature, were heard in most cases. The liver was just palpable in several patients, and the tip of the spleen was thought to be palpable in three. No abnormality was found in the nervous system in any case. In the whole series of cases included in this report there was a considerable number of patients who gave a history that they had at some time been told they had 'heart trouble' and been treated without benefit with digitalis.

*Laboratory investigations.* Including two patients with moderate anaemia, attributed to benzol poisoning, who had not consulted a doctor, but were found to be anaemic in the course of a routine examination, the average blood count when the patients first entered the hospital was—red cells 1,700,000 per c.mm., haemoglobin 42 per cent., colour index 1.23, white cells 2,250 per c.mm., mean corpuscular volume 96 cub.  $\mu$ , reticulocytes 4.4 per cent. In stained films the red cells appeared normal in size or slightly larger than normal; there was slight anisocytosis, but little or no poikilocytosis. Immature cells were seen in the films of two of the 11 cases. Free hydrochloric acid was present in the stomach, usually only after the administration of alcohol or histamine, in the five cases in which a test meal was performed. In nine cases haemolysis of red cells began in an average concentration of 0.43 per cent. of saline, and was complete at 0.37 per cent., the extreme limits being from 0.50 to 0.30 per cent. (average values in 17 control observations on normal subjects 0.44 to 0.33 per cent.; limits 0.46 to 0.28 per cent.). The fragility of the red cells in these cases was therefore practically normal. The icterus index was above normal values and varied from 6 to 15 in four cases out of the six in which it was determined.

*Clinical course.* The average duration of the illness in the five patients who died was one year and five months; two died within 10 weeks of the onset and one survived for two years and eight months. Including the two cases with mild anaemia attributed to benzol poisoning, five patients in this group had remissions. The subject of remissions will be dealt with in some detail in a later section. In the more severe cases there was increasing fever for which no cause other than the anaemia could generally be found. Bleeding usually became more severe and increasingly difficult to control, and

transfusions had progressively less good effect. One patient in this group developed a necrotic ulcer of a tonsil, and one had a necrotic ulcer of the perineum and a small one of the lip. The immediate cause of death appeared most commonly to be a pulmonary complication, such as pulmonary oedema, bronchopneumonia, and, in one case, a lung abscess.

### *Case Reports*

Refractory anaemia with hypocellular marrow, or aplastic anaemia.

*Case 8.* An American University student, 18 years of age, had an attack of sore throat and fever, diagnosed as influenza, five months before the time of her admission to the Rockefeller Institute Hospital. She had had no previous illnesses other than childhood fevers, and her family history appeared unimportant. Her diet had been normal, and there was no known exposure to toxic substances, except that at the time of her first complaint she was given a small quantity of a compound containing phenacetin. Three months later she had a further attack described as tonsillitis and peritonsillitis, from which she recovered in 10 days. Four weeks later she had a further similar attack. After one week her throat had improved, but her fever increased and she had several rigors. She was found to be anaemic, with haemoglobin 50 per cent. and white cell count 2,000 per c.mm., and slight oozing from the gums occurred. In the next three weeks she received three transfusions.

*Laboratory investigations.* Blood count on admission, red cells 2,700,000 per c.mm., haemoglobin 52 per cent., colour index 0.96, mean corpuscular volume 80 cub.  $\mu$ , reticulocytes 2.0 per cent., platelets 12,000 per c.mm., white cells 2,050 per c.mm.; differential count, polymorphonuclear leucocytes 22 per cent., lymphocytes 75 per cent., monocytes 3 per cent. Icterus index 6. Fragility of red cells in saline normal. Stools, occult blood test positive. Blood culture negative. Urobilinogen output in 24 hours, urine 1.8 mg., stools 149 mg.

*Sternal marrow biopsy.* Marrow almost completely aplastic, with some pink areas and some areas of haemorrhage. In between the fat cells are small groups of cells, mostly resembling lymphocytes, and a few normoblasts. No megakaryocytes seen.

*Further course.* The patient became progressively weaker with high fever and tachycardia, increasing bleeding from gums and into the skin, multiple retinal haemorrhages, and finally gross haematuria. She developed some necrosis of the right tonsil, and clinical and radiological evidence of a small abscess of the right lung. Her haemoglobin fell, in spite of five transfusions and treatment with ascorbic acid and liver extract. Her icterus index increased to 15, and she died four weeks after her admission to hospital and six months after the onset of her illness.

*Summary of autopsy findings.* Well nourished girl. Extensive subcutaneous ecchymoses, especially about right eye. Extensive slough of right tonsillar fossa. Numerous haemorrhages into serous membranes, gastrointestinal tract, and peripelvic tissue of kidney. Haemorrhagic bronchopneumonia, with small right lung abscess. Spleen normal in size, consistency, and on cut section. Liver normal in size, with yellow areas around central veins. Sternal, costal, and femoral marrow almost completely fatty.

*Summary of microscopical findings.* Marrow sections show almost complete aplasia, with a few lymphocytes and very occasional myelocytes between the fat cells. Liver, advanced central necrosis. Spleen, slight hemosiderosis

and extramedullary erythropoiesis. Lymphatic glands and other organs show no abnormality.

Refractory anaemia with hypocellular marrow, aplastic anaemia, with rapid course.

*Case 9.* A married Scottish housewife, aged 64 years, who had lived in America for 47 years, first complained of weakness, dyspnoea, and palpitation two months before admission to hospital. A brother died at the age of 50 years of pernicious anaemia; the family history otherwise seemed unimportant. There was no history of exposure to toxic substances. The patient had been subject to attacks of bronchitis for 20 years, and four years previously had been said to have high blood-pressure. Soon after the first symptom of her present illness she was found to be anaemic. Purpura and small ulcers of the mouth appeared. Treatment with liver extract and a blood transfusion produced only temporary improvement.

*Physical examination.* Temperature 100.2° F., pulse 96. Obese, very pale, elderly woman. Numerous petechiae on skin and mucous membranes. No jaundice. Recent retinal haemorrhages. Tongue and throat normal. Chest, crepitations at bases and widespread rhonchi. No abnormality in cardiovascular system. Liver, spleen, and lymphatic glands not felt. No neurological abnormality.

*Laboratory investigations.* Blood count on admission, red cells 1,500,000 per c.mm., haemoglobin 28 per cent., colour index 0.9, mean corpuscular volume 80 cub.  $\mu$ , platelets 42,000 per c.mm., white cells 1,000 per c.mm.; differential count, polymorphonuclear leucocytes 9 per cent., lymphocytes 86 per cent., monocytes 2 per cent., eosinophils 3 per cent. Stools, occult blood test negative. Radiograph of chest and barium meal, no abnormality.

*Further course.* The patient's haemoglobin fell to 13 per cent. She developed acute pulmonary oedema rather suddenly two weeks after she entered hospital, and died ten weeks after her first definite complaint.

*Summary of autopsy findings.* Very obese, pale, elderly woman. Numerous petechiae and small ecchymoses. Small ulcerations on lips, tongue, and buccal mucosa. Bright yellow subcutaneous fat. Subpericardial haemorrhages. Oedema and congestion of lungs, with haemorrhage into alveoli. Fatty infiltration of liver (1,170 gm.). Soft wrinkled spleen (115 gm.) with little pulp. Small ulcers in terminal ileum. Atheroma, with some ulceration, of aorta. No enlarged lymphatic glands. Sternal, costal, and femoral marrow appears completely fatty with some red colour.

*Summary of microscopical findings.* Marrow consists almost entirely of fat. There are a few strands of cells between the fat, consisting chiefly of lymphocyte-like cells with a few groups of normoblasts. Liver, moderate fatty infiltration. Lungs, mononuclear cells and fibrin in alveoli and bronchi. No abnormality seen in spleen, lymphatic glands, or other organs.

Refractory anaemia with hypocellular marrow, aplastic anaemia, with evidence of an increased rate of haemolysis and rapid course.

*Case 10.* A married Irishman, aged 54 years, previously healthy, had suffered from high blood-pressure for 12 years. Four months before admission an attack of cerebral haemorrhage caused him to give up his employment as a fireman in an oil company. Six weeks later a further attack was diagnosed as coronary thrombosis, and he recovered in about six weeks. About two months before he was admitted, he complained of weakness, pallor, shortness of breath, and swelling of the feet. His haemoglobin was

30 per cent., his skin was unusually yellow, and he developed purpura and bleeding from the gums. He was treated with liver extract by injection and one transfusion, with no more than temporary benefit.

*Physical examination.* Temperature 96·8° F., pulse 76. Obese elderly man; dyspnoeic on exertion and at times irrational. Pallor and slight jaundice of skin and sclerae. Recent and old retinal haemorrhages. Slight atrophy of mucous membranes at edges of tongue. Barrel-shaped chest with crepitations at bases of lungs. Peripheral arteries thickened. Heart enlarged. Blood-pressure 120/65. No enlargement of liver, spleen, or lymphatic glands. No neurological abnormality.

*Laboratory investigations.* Blood count on admission, red cells 1,200,000 per c.mm., haemoglobin 30 per cent., colour index 1·28, mean corpuscular volume 104 cub.  $\mu$ , reticulocytes 3·6 per cent., white cells 2,100 per c.mm. Icterus index 15. Fragility of red cells in saline, haemolysis begins at 0·48 per cent. and is complete at 0·38 per cent. (control 0·44 to 0·32 per cent.). Urobilinogen output in 24 hours, stools 463 mg., urine urobilinogen increased (no bile present).

*Sternal marrow biopsy.* Bone spicules appear diminished. Marrow hypocellular, but patchy. Some areas almost completely fatty, some of haemorrhage, and some of quite cellular marrow with erythroblasts, normoblasts, sometimes in conspicuous groups, myelocytes, and polymorphonuclear leucocytes.

*Further course.* Patient remained irrational and slightly jaundiced, and had slight bleeding from the gums and purpura. His haemoglobin fell to 19 per cent., in spite of transfusions, and he died of acute pulmonary oedema two weeks after admission to hospital and 10 weeks after his first symptoms.

*Summary of autopsy findings.* Obese elderly man with oedema of ankles. Lemon-yellow skin and very yellow subcutaneous fat. No enlarged glands. Pericardial cavity obliterated by fibrous adhesions. Oedema and congestion of lungs. Heart enlarged with evidence of old coronary infarction. Liver (1,270 gm.) yellowish-brown and fatty. Spleen (200 gm.) on section shows prominent follicles and little pulp. Vertebral, costal, sternal, and femoral marrow fatty with scattered red areas.

*Summary of microscopical findings.* Marrow hypocellular with some diffusely, but rarely densely, cellular areas. Compared with biopsy section there is a relative increase of lymphocyte-like and other immature cells; few erythroblasts, eosinophil myelocytes, normoblasts, and polymorphonuclear leucocytes. No megakaryocytes seen. Heart, sclerosis of coronary arteries with increased interstitial tissue in cardiac muscle. Spleen, congestion and sclerosis of arteries of germ centres, slight haemosiderosis, no other abnormality. Haemorrhagic exudate in lung alveoli.

### 5. *Refractory Anaemia with Immature Cellular Marrow, or Chronic Granulocytopenia*

*Pathology.* Of the seven patients who had this type of marrow on biopsy examination, six were examined *post mortem*. In two cases a post-mortem examination only was made and in five more the marrow was of the partly mature cellular type at biopsy and of the immature cellular type *post mortem*. In all, therefore, 13 cases were examined *post mortem*. Some sections from an incomplete post-mortem were available in a fourteenth. In most cases the bodies were well nourished; in two cases they were described

as poorly nourished and in one, that of a boy with haemochromatosis, as emaciated. The skin and mucous membranes were pale and in a minority of cases slightly yellow; in one case there was considerable jaundice. Purpuric haemorrhages were present in four of the 13 cases, but were not usually as numerous as in those in the group with hypoplastic marrow. There was slight mottled brown pigmentation, especially of the arms and neck, in two cases and considerable pigmentation in a third. The nails were normal, and slight atrophy of the papillae at the edge of the tongue was present in one case only.

In eight of the cases, areas of cellulitis or necrotic ulceration were present at the time of death. These were often multiple, and appeared as greyish-black, ragged, foul-smelling, sloughing areas. In four cases extensive lesions of this type were present in the mouth and pharynx, involving, in different cases, the lips, gums, tongue, epiglottis, pillars of the fauces, and one or both tonsillar fossae. One of these patients had in addition a necrotic ulcer of the right shoulder and small ulcers in the terminal ileum; another had an ulcer in the larynx. In three cases there were extensive sloughing areas in one or other ischio-rectal fossa, and in one of these cases in addition a cellulitis of the neck, which had been incised and the edges of the incisions had become necrotic. In one case there was a draining abscess behind the right ear. In two other cases there were in one multiple small ulcers of the oral mucous membrane and in another a longitudinal ulcer of the lower oesophagus.

Internal haemorrhages were present in three of the 13 cases, in the pericardium, epicardium, and pleura. Slight enlargement of mesenteric, retro-peritoneal, and mediastinal lymphatic glands (up to  $1.0 \times 1.0 \times 0.5$  cm.) was present in four cases, and was usually associated with haemosiderosis, visible to the naked eye. Microscopically the normal architecture of the lymphatic glands was retained, and the lymph follicles were normal in appearance. The pulp strands were often conspicuous, showing numerous phagocytes containing haemosiderin against a background of pink-staining reticulum, and being almost devoid of other cells; in one case the pulp strands were similarly conspicuous and contained a few giant cells, but no haemosiderin. In two cases, one of them with frank haemochromatosis, there were very large amounts of haemosiderin. In most cases there was slight extramedullary haemopoiesis, indicated by the presence of myelocytes, almost always eosinophil, primary erythroblasts, and normoblasts. In a few cases this change was quite extensive, but it had not the uniform appearance of leukaemic infiltration, nor did it obliterate the normal structure of the gland.

The average weight of the spleen was 235 gm., the largest being 400 gm. and the smallest 130 gm. The spleen was thus slightly or moderately enlarged. The capsule was free from adhesions and showed nothing remarkable. The cut surface was described as dark purple, greyish-red or greyish-purple with abundant pulp and markings less distinct than normal. On microscopical examination the amount of pulp was relatively increased and the lymph follicles were inconspicuous. The pulp was very cellular, but contained

relatively few erythrocytes. In two cases, one the case with haemochromatosis, the spleen had been removed. In nine of the remaining 11 cases haemosiderin was present in slight or moderate amounts, and in seven there was extramedullary haemopoiesis in varying degrees. This was chiefly erythropoiesis with primary erythroblasts and normoblasts scattered in groups about the pulp. There were also occasional eosinophil myelocytes. Exceptionally this change was present quite extensively, but the normal architecture of the spleen was preserved and the changes did not have the characters of leukaemic infiltration.

The average weight of the liver was 1,775 gm., the largest being 2,350 gm. and the smallest 950 gm., and in appearance it was usually pale yellow-brown and fatty. The cut surface was yellow or brown, often with yellow depressed areas around the centres of the lobules. In several cases brown pigment was visible to the naked eye. On microscopical examination there was fatty infiltration in some degree in almost every case; areas of necrosis at the centre of the lobules were present in six cases and this change was severe in three. In another case, that associated with haemochromatosis, there was early portal fibrosis. In the same case the pancreas was firm in consistence and deep in colour, and on microscopical examination showed haemosiderosis and fibrosis. In this case pigment was present also in the marrow, suprarenals, cardiac muscle, and basal layers of the epidermis.

The bone marrow in six cases was deep red in colour and apparently hypercellular in all the bones of the trunk. The femoral marrow appeared less cellular, and fat was visible in the lower half of the femoral marrow in all but one of the six. In three cases the marrow appeared greyish-red and cellular, but with some fat present, in all the bones examined, the femoral marrow containing more fat than the rest. In the remaining four cases the marrow appeared yellow and fatty, with occasional red cellular areas in all the bones examined.

The microscopical appearances of the bone marrow have already been described. Those at autopsy did not differ qualitatively from those at biopsy, but in every case where both biopsy and autopsy sections were available the degree of cellularity was less at autopsy than at biopsy. This point appears important in connexion with the question whether these cases are in any way related to leukaemia. For if, as might be argued, these patients were suffering from an early stage of leucopenic lymphatic leukaemia, in which the leukaemic process was confined to the bone marrow, the opposite change would be expected, namely marrow more cellular at autopsy than at biopsy.

In the remaining organs examined the changes were those seen in any severe anaemia. In one case in the group there was a pyonephrosis, and in another multiple small abscesses in one kidney. In both the affected kidneys there were dense areas of small round cells with very few polymorphonuclears. In a few other cases of this type there were groups of small round cells in the portal systems of the liver, occasional similar groups beneath the

capsule of the kidney, and in one case in the suprarenal. These were cases of patients who had died with infected necrotic areas and, in one case, with a haemolytic streptococcal septicaemia. The changes did not resemble those of leukaemic infiltration.

The immediate cause of death appeared in six cases to be a pulmonary complication, bronchopneumonia in four cases, multiple lung abscesses in one, and severe pulmonary oedema in one. In one case there was a haemolytic streptococcal septicaemia with pink staining of the intima of the blood vessels *post mortem*, and in the case with haemochromatosis diabetes appeared to be a contributing factor. In the remaining cases no cause of death was apparent other than anaemia and infected necrotic lesions.

*Clinical findings.* Twelve patients had marrow of this type at biopsy, or at autopsy where no biopsy was performed. Five were female and seven male. The average age at the onset of symptoms was 48 years, the oldest being 63 and the youngest 19 years. The family histories appeared unimportant.

It was noticeable that a number of patients in this group had been in poor health for some time before the onset of their symptoms of anaemia, and several of them had therefore taken a variety of medicines. Two had asthma, two had suffered for years from dyspepsia, one had apparently psychogenic headaches, and one was troubled by nervousness and recurrent nervous breakdowns. One had had a cholecystectomy for gall-stones and one had an attack of gall-stone colic while in hospital. One had exophthalmic goitre. Three had recently had infections, one pyelitis for some years, one an otitis media, and one an attack of influenza followed by an infected antrum and bilateral acute mastoiditis. For different reasons three of these patients had been living on restricted diets. Of the 12 patients, two had at some time been exposed to benzol, one was a therapeutic radiologist who had undoubtedly been careless about exposing himself to radiation, one had used a paraphenylene diamine hair dye, and four were known to have taken excessive amounts of medicine. In one case the medication consisted of acetanilide and phenobarbitone, in three others a variety of drugs, probably including analgesics of the same type, had been taken for several years, but no exact information could be obtained. In one of them (Case 14) a temporary but complete remission appeared to follow the cessation of all medication, and a subsequent and fatal relapse followed the administration of an unknown amount of allonal. In the second an apparent sensitivity to allonal was demonstrated in hospital, and the third developed fever and necrotic lesions of the gums and oral mucous membranes after a single dose of pyramidon. Though the evidence given here is perhaps no more than suggestive, it seems possible from this and from other cases seen by one of us (C.P.R.) in consultation and not included in this report that the association of inadequate diet, excessive medication, and the development of refractory anaemia of this and other types is significant.

The presenting symptoms varied. In three cases there was weakness and dyspnoea, in two the first symptoms were fever with a rigor, and in several

sore throat with fever and swollen cervical glands. One patient complained of sore gums which were diagnosed as trench mouth, and in one the removal of a polyp from the soft palate was followed by an extensive slough of the roof of the mouth. In one fever with a rigor, diagnosed as influenza, was followed by infection of the tip of the nose, nostrils, and face, and cervical adenitis. Four patients had bleeding from the gums and two from the nose; bleeding was therefore less prominent in this group than in the others, and purpura was present in small degree before admission in only one case. Two patients had temporary and partial remissions before admission, one apparently after liver extract therapy.

On examination these patients were usually pale and sallow, and irregular fever, often high and associated with rigors, was present in almost every case. Purpura and bleeding occurred, but were exceptional. Retinal haemorrhages were seen in five of the 12. The most prominent features of these cases were recurrent infections and necrotic ulcerations. These included sore gums, ulcers of the mucous membranes of the mouth, infected throats, and necrotic ulcers of the tonsils, skin, and perineum.

Slight atrophy of papillae at the edges of the tongue was present in one case. A haemic murmur was heard in about half of the cases. Enlarged lymphatic glands were not seen except in association with infections. The liver was just palpable in six cases and the tip of the spleen in two. No neurological abnormality was discovered in any case.

*Laboratory investigations.* The average blood count in these 12 patients on admission was red cells 2,200,000 per c.mm., haemoglobin 50 per cent., white cells 1,850 per c.mm.; differential count, polymorphonuclear leucocytes 20 per cent., platelets 79,000 per c.mm., mean corpuscular volume 102 cub.  $\mu$ , reticulocytes 1.8 per cent. In stained films the red cells showed some macrocytosis and anisocytosis, but little poikilocytosis. Immature white cells, myeloblasts, and myelocytes were present in small or moderate numbers in the films of the 12 cases, and normoblasts in three. In one case, that attributed to X-irradiation, there were up to 35 per cent. of atypical mononuclear cells, probably immature white cells, and up to 55 immature red cells per 100 white cells in different films. Free hydrochloric acid was present in the stomach, usually only after the administration of histamine or alcohol, in six of eight patients in whom this examination was made. The icterus index determined in six patients gave values above normal and varying from 7 to 20 in four. The red cell fragility determined in one patient was within normal limits.

*Clinical course.* The average duration of the illness in the 11 patients who died was one year and seven months. This figure is misleading since it is raised considerably by the long course of two patients who had temporary remissions and whose marrow was not examined during life. The average duration of the illness in the eight patients who had this type of marrow at biopsy examination during life was 10 months. Only one patient in this group (Case 34) had a remission which lasted for any considerable length of time.



The illness in these patients usually took a severe and often distressing course. Bleeding occurred occasionally, but was much less prominent than in the previous group. There was often high fever and occasionally rigors. Infections or necrotic ulcerations occurred in eight of the 12 cases and were often multiple. They included in different cases severe tonsillitis, necrotic ulceration of the tonsils, cervical adenitis, and cellulitis of the neck, sloughing of incisions made in areas of cellulitis, simple or necrotic ulcerations of the face, tongue, lips, gums, oral mucous membrane, and the skin of the back, abscesses after injections, a paronychia, and three necrotic lesions of the perineum. These began as indurated, dull red, painful swellings on one or other side of the anus, and very slowly increased in size. If incised, even after a considerable period of waiting, a thin serosanguineous fluid exuded, but little or no pus was found. If no opening was made the swellings discharged spontaneously; in either case the edges became necrotic, and usually the rectum was involved and a fistula appeared. Numerous organisms, usually of a saprophytic type, could be cultured from the necrotic areas, but in most cases necrosis rather than infection appeared to be the primary process.

In comparison with the other groups, anaemia in these patients remained less noticeable than granulocytopenia throughout the course of their illness. Thus, up to the time of the last count before death the percentage of haemoglobin remained above 50 in two and above 30 in six. Granulocytopenia on the other hand was often progressive, and in one case shortly before death no leucocytes were found in an examination of 50 fields of a stained film.

The immediate cause of death appeared most commonly to be lung complications, bronchopneumonia in four cases, multiple lung abscesses in one, and infarcts in two. Another patient developed respiratory obstruction from cellulitis of the neck and died during an operation for tracheotomy. One developed a haemolytic streptococcal septicaemia and one a bacillus coli septicaemia.

### *Case Reports*

Refractory anaemia with immature cellular marrow.

*Case 11.* A German shoemaker, 28 years of age, first complained of sore mouth and gums, diagnosed as trench mouth, four months before admission to hospital. He had had no illnesses since infancy, except that one year previously a doctor had told him that he had high blood pressure. The family history appeared unimportant and his diet had been adequate. He was given three injections of neosalvarsan before admission to hospital. After his first complaint he remained unwell with pallor, loss of appetite, and some loss of weight. Three weeks before admission he developed a peritonissillar swelling with high fever. The swelling was incised, but no pus was obtained.

*Physical examination.* Temperature 101.2° F., pulse 104. Well developed and well nourished. Tongue normal, few petechiae on hard palate. No other petechiae, and no retinal haemorrhages. Swelling and oedema of right

tonsil, which was covered with yellow necrotic material. Swelling of right cervical lymphatic glands, but no other enlarged lymphatic glands. Soft systolic murmur heard over praecordium. Blood-pressure 118/55. Liver and spleen not felt. No neurological abnormality.

*Laboratory investigations.* Blood count on admission, red cells 2,300,000 per c.mm., haemoglobin 50 per cent., colour index 1.1, mean corpuscular volume 99 cub.  $\mu$ , platelets 146,000 per c.mm., white blood cells 1,500 per c.mm.; differential count, polymorphonuclear leucocytes 14 per cent., lymphocytes 80 per cent., myelocytes 6 per cent. Test meal, free hydrochloric acid present after alcohol and histamine. Wassermann reaction negative.

*Sternal marrow biopsy.* Normal bone spicules. Section consists of areas of densely packed cells, separated by areas of pink structureless material. The cellular areas consist almost entirely of early primary erythroblasts. The only other cells present are occasional myelocytes and very occasional polymorphonuclear leucocytes. There is some haemosiderosis.

*Further course.* For a short time after admission the patient's general condition improved. His throat healed and his temperature became normal, but his blood count slowly decreased. Subsequently a series of small yellow ulcers with red inflamed margins appeared on the tongue and mucous membranes. One of these on the inside of the lower lip enlarged and developed a black necrotic centre. There was considerable surrounding induration and fever to 102° or 103° F. The necrotic area was removed as soon as a line of demarcation had formed, and the area had healed and the temperature returned to normal one month after the lesions first appeared. Three and a half months after admission the patient had a brief attack of upper right abdominal colic, apparently gall bladder colic. Following this an abscess developed in the left buttock at the site of a liver injection, again with high fever. About 30 c.c. of pus was evacuated on incision of the abscess. Severe haemorrhage occurred from the cavity and made transfusion necessary; the cavity eventually healed. Further painful indurated ulcers on the tongue and oral mucous membrane appeared in the sixth and seventh months in hospital, and the abscess of the left buttock recurred and was reopened. At this time there was some oozing of blood from the gums, easily controlled by the application of silver nitrate. In the eighth month there was a further necrotic ulcer below and posterior to the left tonsil which caused dysphagia; the ulcer healed, but recurred in the ninth month. Cultures from the throat grew *Haemophilus haemolyticus*, *Pneumococcus*, *Streptococcus viridans* and indifferent *Streptococci*. On this occasion the ulcer enlarged, causing pain in the left cheek and ear, dysphagia, and haemorrhage. The patient then developed laryngeal obstruction, which made necessary an emergency tracheotomy. After this, signs of pneumonia appeared in the right lower lobe, and he died five days after the tracheotomy, and 13 months after his first symptom.

*Summary of autopsy.* Moderately well nourished. Area of necrotic ulceration beneath left tonsil extending towards epiglottis. Recent tracheotomy wound with necrotic edges. Subcutaneous fat bright yellow. Right bronchopneumonia. Slight enlargement of mediastinal lymphatic glands only. Ulcer (0.5 cm. diam.) on anterior surface of larynx. Spleen (195 gm.) with dark-purple cut surface and normal markings. Liver (1,900 gm.) with mottled brownish-red cut surface and normal markings. Gall bladder enlarged (15 cm. in length) and thin-walled. No other abnormality in biliary tract. Marrow of sternum, vertebrae, ribs, and femur deep-red and without fat.

*Microscopical findings.* Marrow from sternum, vertebrae, and femur diffusely, but not densely, hypercellular. There is little remnant of normal architecture. Otherwise the appearances are very similar to those of the biopsy section, with an increase in the number of late primary erythroblasts. Very occasional myelocytes. Few megakaryocytes. No haemosiderosis. Lungs, bronchopneumonia. Liver, severe fatty infiltration round central veins. Spleen, moderate haemosiderosis and extramedullary erythropoiesis. Slight erythropoiesis in lymphatic glands, but no evidence of leukaemic infiltration.

*Case 12.* A married Finnish carpenter, 58 years old, first noticed some loss of strength, weight, and appetite about four months before admission. His only previous illnesses were childhood fevers. He had worked for 15 years in a rubber factory exposed to volatile organic solvents, but had not been so exposed for four years before he became ill. He ate fresh fruit and vegetables daily, but his consumption of meat had been considerably restricted for the previous six years. For three or four years he had done very little work. Two months before admission he complained of a sore throat. A doctor removed what was thought to be a polyp from his soft palate. A sloughing ulcer developed at the site of this operation and the patient was referred to the Memorial Hospital. There was then a large necrotic mass involving the soft palate, uvula, and tonsils, together with anaemia, leucopenia, and fever. Several biopsies showed no sign of cancer. The patient was referred to the Rockefeller Institute Hospital.

*Physical examination.* Temperature 101.6° F., pulse 108. Well developed and well nourished. Skin rough, especially over arms, abdomen, and knees. Gums spongy and retracted; tongue normal. Greyish-yellow necrotic mass with surrounding oedema involving soft palate, pillars of fauces, and tonsils, almost obliterating nasopharynx. A few enlarged lymphatic glands in neck, none elsewhere. Chest and heart normal. Blood pressure 134/80. Liver and spleen not felt. No neurological abnormality.

*Laboratory investigations.* Blood count on admission, red cells 2,900,000 per c.mm., haemoglobin 70 per cent., colour index 1.2, mean corpuscular volume 103 cub.  $\mu$ , white blood cells 1,250 per c.mm.; differential count, polymorphonuclear leucocytes 28 per cent., lymphocytes 70 per cent., eosinophils 1 per cent., myelocytes 1 per cent. Plasma bilirubin 1.3 mg. per 100 c.c. Wassermann reaction negative. Throat culture, negative for haemolytic streptococcus. Urine bright red, but after being passed turned brown on standing; on spectroscopy strong absorption bands of porphyrin present (there was no other evidence of porphyria in this patient).

*Sternal biopsy.* The section shows marrow with normal architecture, except for some pink structureless areas. It appears slightly more cellular than normal. The cells are almost entirely late primary erythroblasts with some normoblasts and myelocytes. The normoblasts have deeply basophil cytoplasm. Megakaryocytes in about normal numbers.

*Further course.* The patient's fever increased. His haemoglobin decreased to 54 per cent., and his white cell count to 500 per c.mm. The necrotic area in his throat increased in size, and he died two weeks after admission to hospital and 4½ months after his first symptoms.

*Summary of autopsy findings.* Well nourished. Skin lemon-yellow colour; subcutaneous fat bright yellow. Yellowish-grey foul-smelling necrotic area involving soft palate, tonsils, and pillars of fauces. Partially healed sternal biopsy incision. Multiple abscesses of lungs. Spleen (400 gm.) moderately

enlarged and soft, with cut surface showing deep purple haemorrhagic areas (5 cm. diam.) separated by tissue with normal splenic markings. Liver (2,350 gm.) with brown cut surface and irregular light-yellow areas at centres of lobules. Ulcers (0.5 cm. diam.) in lower ileum. Slight enlargement of mesenteric lymphatic glands, but no other enlarged lymphatic glands. Sternal, costal, and vertebral marrow deep-red and apparently cellular. Femoral marrow similar in upper half, partly fatty in lower.

*Summary of microscopical findings.* Marrow, except from femur, similar to that of biopsy section, but perhaps a little less cellular, consisting chiefly of late primary erythroblasts. Section from femoral marrow less cellular, with considerable amounts of fat. Lungs, bronchioles filled with exudate and masses of bacteria; one small abscess. Spleen, congestion, slight haemosiderosis, and extramedullary erythropoiesis. Liver, central fatty infiltration. Lymphatic glands normal. No evidence of leukaemic infiltration.

*Case 13.* A married American cotton grower 52 years of age, who had had no illness since childhood, first complained of pallor and shortness of breath 11 months before his admission. His family history seemed unimportant, there was no known exposure to chemicals, and he had always eaten an unrestricted normal diet. His illness was first thought to be pernicious anaemia and he was treated with liver. Subsequently he was three times in hospitals and had various treatments including iron, liver extract, ventriculin, Fowler's solution, and 21 blood transfusions, with no permanent benefit. Ten days before admission his gums began to bleed, his tongue became sore, and he complained of increasing pain in both legs and the right hip.

*Physical examination.* Temperature 98.4° F., pulse 92. Well nourished. No jaundice. One ecchymosis on right thigh, single haemorrhage in right fundus. Spongy red bleeding gums. Tongue, salmon-pink in colour and tender. No atrophy of papillae. Blood pressure 105/58. Loud systolic murmur at cardiac apex. Liver just felt; spleen not felt. No enlargement of any lymphatic glands. No neurological abnormality.

*Laboratory investigations.* Blood count on admission, red cells 2,350,000 per c.mm., haemoglobin 56 per cent., colour index 1.02, mean corpuscular volume 94 cub.  $\mu$ , reticulocytes 0.4 per cent., platelets 92,000 per c.mm., white blood cells 1,000 per c.mm.; differential count, polymorphonuclear leucocytes 19 per cent., lymphocytes 60 per cent., eosinophils 1 per cent., basophils 0.5 per cent., myelocytes 15 per cent., myeloblasts 4.5 per cent. Test meal, achlorhydria after alcohol and histamine.

*Sternal biopsy.* Section shows normal bone spicules and focal areas of considerably hyperplastic marrow in solid sheets separated by extensive areas, some of pink structureless material and some of haemorrhage. The cellular areas consist almost entirely of primary erythroblasts, mostly early and a few late. There are a few normoblasts with basophil cytoplasm. No myelocytes or megakaryocytes seen.

*Further course.* The patient remained afebrile for some weeks. He then developed a peri-anal abscess and a hard swelling on the right side of the neck, with irregular fever. The swelling in the neck was incised, but the margins of the incision sloughed. Bleeding from the gums became increasingly profuse, and he had considerable nose bleeding. He obtained little benefit from transfusions, which were usually followed by rigors. Finally he complained of severe pain in the chest, and a friction rub was heard. He died apparently from inhalation bronchopneumonia nine weeks after admission and 13 months after his first symptoms.

*Summary of autopsy report.* Well nourished man, with numerous ecchymoses on the skin. Irregular swelling of right side of neck, with two openings lined by grey, apparently gangrenous, tissue. Subcutaneous fat unusually yellow. Small blood-stained pleural effusions. Lungs, bronchopneumonia with early abscess formation. Spleen (130 gm.) with smooth purple cut surface and normal markings. Liver (1,400 gm.) with uniform brown cut surface and normal markings. Slight enlargement (to  $1.0 \times 1.5$  cm.) of para-aortic lymphatic glands; no other enlarged lymphatic glands. Sternal and vertebral marrow bright red and abundant; femoral marrow reddish-yellow and appears to contain a considerable amount of fat.

*Microscopical findings.* The femoral marrow has normal architecture and contains fat. The remaining sections are diffusely hypercellular with some dense cellular areas. The predominant cells are early primary erythroblasts as in the biopsy sections. There are also some normoblasts. There are a very few myelocytes, but no other sign of leucopoietic cells. Moderate haemosiderosis is present. No megakaryocytes seen. The lungs show bronchopneumonia. There is moderate haemosiderosis of the liver, spleen, and lymphatic glands, and extramedullary erythropoiesis in the spleen. There is no leukaemic infiltration in any organ.

*Case 14.* A married American woman, 43 years old, was first found to be anaemic after an attack of influenza and operations for drainage of the left antrum and right mastoid. She was given a transfusion, and was apparently well for 18 months. She then required three transfusions in connexion with a left mastoid operation. After that time she had some anaemia, but no symptoms attributable to it. Seven months before admission she again had 'influenza' and a further transfusion. Since then she had taken liver extract by mouth and later by injection, without significant improvement in her blood count. Her family history appeared unimportant, her diet had been good, and there was no history of exposure to toxic substances. Menstruation had been regular, but had diminished in amount during her present illness.

*Physical examination.* Temperature  $99.1^{\circ}$  F., pulse 78. Well nourished woman with greying hair. Moderate pallor; some atrophy of papillae of tip and edges of tongue. Small adenoma of right lobe of thyroid. Chest and heart normal. Liver, spleen, and lymphatic glands not enlarged. No neurological abnormality.

*Laboratory investigations.* Blood count on admission, red cells 2,500,000 per c.mm., haemoglobin 59 per cent., colour index 1.2, mean corpuscular volume 110 cub.  $\mu$ , reticulocytes 0.8 per cent., white blood cells 1,500 per c.mm.; differential count, polymorphonuclear leucocytes 8 per cent., lymphocytes 89 per cent., monocytes 3 per cent. Macrocytosis, anisocytosis, little poikilocytosis, some punctate basophilia. Icterus index 2.5. Wassermann reaction negative. Test meal, free hydrochloric acid present after alcohol and histamine. Radiographs of chest and sinuses, barium meal and barium enema, no abnormality. Basal metabolic rate +4 per cent.

*Further course.* Patient had slight fever during her 10 weeks stay in hospital. She developed an abscess of the gums of the left lower jaw, followed by a right paronychia, an abscess of the face in front of the left ear which required incision, and a troublesome necrotic ulcerated area in the left lower gums, associated with considerable swelling and induration. Her blood count fell slowly, and the effect of transfusions lasted a considerable time. She was treated intensively with liver preparations. The

reticulocyte count varied between 0.2 and 3.2 per cent. Blood count on discharge, red cells 2,900,000 per c.mm., haemoglobin 55 per cent., white cells 1,500 per c.mm. Shortly after discharge, a few myelocytes and myeloblasts were found in the differential count. Six months after she was discharged, and shortly after all medicines, including some barbiturates, had been discontinued, she had a complete remission, her blood count two months later being red cells 5,000,000 per c.mm., haemoglobin 98 per cent., colour index 1.0, white cells 9,200 per c.mm.; differential count, polymorphonuclear leucocytes 65 per cent., eosinophils 3 per cent., lymphocytes 27 per cent., monocytes 3 per cent. She remained well for a year. She was then in Jamaica and complained of malaise and sore throat, for which she was given a large but unknown amount of allonal. Her sore throat and fever persisted and she returned to New York. Her blood count at this time was, red cells 3,400,000 per c.mm., haemoglobin 68 per cent., white cells 2,000 per c.mm.; differential count, polymorphonuclear leucocytes 27 per cent., lymphocytes 71 per cent., monocytes 2 per cent. She developed an ischiorectal abscess which was incised, and the area affected sloughed. In spite of transfusions she gradually became weaker and died in coma four months after the relapse and  $5\frac{1}{2}$  years after her first symptom.

A limited autopsy was performed.

*Summary of microscopical findings.* Marrow hypocellular, large areas with very few cells. Some areas diffusely, but not densely, cellular. The cellular areas consist almost entirely of lymphocyte-like cells with a few haemocyto blasts, normoblasts, and plasma cells. There is therefore little maturation, and what there is is all erythropoietic in character. Liver, central atrophy, with occasional lymphocytes. No leukaemic infiltration. Extramedullary erythropoiesis in spleen and lymphatic glands.

#### 6. *Refractory Anaemia with Fibrosis, Sclerosis, and Giant Cell Hyperplasia of the Marrow, or Myelosclerosis*

*Pathology.* Post-mortem examinations were performed on only two cases with anaemia of this type, and the findings are described in the individual case reports.

*Clinical findings.* Four patients were seen with this type of marrow during life. One was female and three were male. The average age at the onset of symptoms was 35 years, the youngest being 28 and the oldest 41 years. The family histories of these patients appeared unimportant, as did their previous illnesses. None gave a history of a poor diet. One for five years had been exposed daily to nitrous oxide, and for three days about a month before the onset of his symptoms to nitric oxide; at this time he was dizzy and nauseated.

The presenting symptoms were those of any anaemia. One patient, a woman of 28 years, was two months pregnant when she complained of weakness and was found to be anaemic. Another with a history extending over six years was found five years before admission to have enlarged cervical and axillary lymphatic glands. One of these was removed and was said to show 'chronic lymphadenitis'. The patient was given 18 X-ray treatments and the glands disappeared. One patient had had a sudden

blurring of vision and was found to have retinal haemorrhages. Apart from this there was no history of bleeding or purpura in any of the cases.

On admission all four patients appeared adequately nourished. Three had considerable fever and one was afebrile. They were pale and in two of them their pallor had a subicteric tint. Retinal haemorrhages were present in three and one had slight bleeding from the gums. One patient showed a considerable diffuse pigmentation, but this was thought to be racial. At the time of admission the liver was just palpable in three cases and was below the umbilicus in a fourth; the spleen was not felt in one, was just palpable in two, and below the umbilicus in the fourth. One patient had enlarged lymphatic glands (up to about 1.5 cm. in diam.) in the axillae and groins. No neurological abnormality was present in any case.

*Laboratory findings.* The blood counts in the four cases on admission are shown in the table on p. 190. The average of the counts was—red cells 1,150,000 per c.mm., haemoglobin 27 per cent., white cells 2,013 per c.mm., polymorphonuclear leucocytes 38 per cent. Immature white cells were present in the films in four cases and normoblasts in two. The platelets counted in two cases were 300,000 and 308,000 per c.mm. and the reticulocytes 0.4 and 3.7 per cent. The average mean corpuscular volume in the four cases was 101 cub.  $\mu$ . The icterus index was normal in the two cases in which it was determined. Free hydrochloric acid was present in the gastric juice in all of the three cases in which a test meal was performed. Haemolysis of the red cells in saline was normal in the one case in which it was determined.

*Further course.* The duration of the illness in the four patients was 7 years, 2½ years, 16 months, and 10 months. No remissions occurred, and no treatment, other than transfusions, appeared to have any effect. Three of the patients had considerable fever, for which in two of them no cause other than anaemia could be found. One patient had repeated infections of the neck, face, a finger, and a thumb, followed by ulceration of the tip of the tongue and gingivitis. Another shortly before death developed a necrotic ulcer on the inner surface of the lower lip. Excluding the patient with enlarged lymphatic glands on admission, there was no enlargement of lymphatic glands except in association with infections. The liver and spleen in three patients increased progressively in size so that when last seen the liver was palpable up to 6 cm. below the costal margin, and the spleen could be felt below the umbilicus in all three cases. The anaemia remained severe and myelocytes were seen frequently in the films. The immediate cause of death appeared to be bronchopneumonia in two cases, and vomiting in a third.

### Case Reports

Refractory anaemia with fibrosis, sclerosis and giant cell hyperplasia of the marrow, myelosclerosis.

*Case 15.* A married woman of Turkish and Jewish extraction, who was born in Buenos Aires, first complained of shortness of breath at the age of

21 years, one year before the time of her admission to the Rockefeller Institute Hospital. Her family history and previous history seemed unimportant. She had had one miscarriage, and two children were living and well. At the time of her first symptom she was two months pregnant. She was found to have a severe anaemia with haemoglobin 19 per cent. and red cells 900,000 per c.mm. She was given liver, iron, and numerous transfusions, and was delivered at term of a normal baby girl. Five days after delivery a severe uterine haemorrhage occurred. She continued to have symptoms of severe anaemia and to be treated with transfusions. On the day before her admission she developed fever and had a rigor.

*Physical examination.* Temperature 100.4° F., pulse 104. Well developed and well nourished. Extremely pale. No haemorrhages. Tongue normal. Haemic murmur. Liver edge just felt. Spleen not felt. No enlarged lymphatic glands. No neurological abnormality.

*Laboratory investigations.* Blood count on admission, red cells 1,230,000 per c.mm., haemoglobin 33 per cent., colour index 1.37, mean corpuscular volume 94 cub.  $\mu$ , white cells 3,100 per c.mm.; differential count, polymorphonuclear leucocytes 31 per cent., lymphocytes 41 per cent., monocytes 16 per cent., myeloblasts 1 per cent., myelocytes 9 per cent., unclassified 2 per cent. Test meal, free hydrochloric acid present after alcohol and histamine.

*Biopsy of sternal marrow.* Bone spicules appear normal. Over the greater part of several sections the marrow spaces are filled with dense fibrous tissue containing in its meshes a few haemopoietic cells. There are a few areas of typical basophil staining sclerosis. In other parts of the sections there are sheets of hypercellular marrow of the partly mature type with haemocyto blasts, erythroblasts, normoblasts, myelocytes, and polymorphonuclear leucocytes. The number of megakaryocytes appears increased.

*Further course.* The patient was an in-patient on eight occasions in the next 18 months. Throughout this time she had slight irregular fever with an occasional rise to 103° or 104° F. She received in all 26 further transfusions. Her illness was complicated by a series of infections, a cellulitis of the right side of the face, a cellulitis and localized abscess of the left side of the neck, from which a haemolytic *Staphylococcus aureus* was grown, two further small abscesses of the left side of the neck, an abscess of the left breast, and an infected finger (from which *Staphylococcus aureus* was grown), and finally an infection of the left side of the face. Shortly after her first admission she was transferred to the Memorial Hospital and given a course of 10 X-ray treatments to the long bones. No improvement followed this procedure. Apart from these recurrent infections the patient's condition changed little to a month or two before her death. After transfusions she was comfortable, but as their effect wore off she had severe symptoms of anaemia. No haemorrhages occurred. The red cell count remained between 1,000,000 and 2,000,000 per c.mm., with a colour index usually slightly above 1.0. The white cell count on most occasions was between 1,000 and 2,000 per c.mm. The percentage of polymorphonuclear leucocytes was below normal, and myeloblasts and myelocytes were constantly present in the films; there were also at times cells, apparently of the leucopoietic series, of bizarre appearance, which defied classification. Sixteen months after her first admission it was noticed that her liver was palpable 4 cm. below the costal margin and her spleen, which on previous occasions had never been more than moderately enlarged, was felt below the umbilicus. There was no enlargement of peripheral lymphatic glands, except for a slight induration of some of those in the neck, after repeated infections in that situation.



Some weeks after this she developed an ulcer of the tip of her tongue, and sore gums. She died 18 months after her first admission, and 29 months after her first symptoms.

*Summary of autopsy findings.* The body was that of a well developed and well nourished woman. Subcutaneous fat more yellow than usual. Hypertrophy of left ventricle. Oedema of lungs. Spleen (1,048 gm.) greatly enlarged and reaching pelvic brim. Outer surface smooth, cut surface shows normal markings and multiple deep red or black areas (to 0.5 cm. in diam.). Liver (2,650 gm.) enlarged, with firm golden-brown cut surface, showing greyish discoloration around portal areas. Left kidney enlarged (270 gm.), cut surface has yellow translucent appearance with indistinct markings. Right kidney, normal in size, with areas of translucent pale tissue on cut surface. Lymphatic glands in all groups normal in size and appearance. Sternal, costal, and vertebral marrow apparently replaced by white fibrous tissue; femoral marrow mottled reddish grey.

*Summary of microscopical findings.* Marrow sections similar to those at biopsy, with areas of fibrosis, areas of partly mature cellular marrow, a conspicuous increase of megakaryocytes, and some haemosiderosis. Lymphatic glands, haemosiderosis and considerable extramedullary erythropoiesis, megakaryocytes also present. Spleen, pulp relatively increased in amount, moderately intense extramedullary haemopoiesis. Liver, fatty degeneration and necrosis at centres of lobules, slight portal fibrosis, haemosiderosis and extramedullary haemopoiesis.

*Case 16.* A Syrian labourer first complained of weakness and dyspnoea at the age of 26 years, three months before the time of his admission to the Rockefeller Institute Hospital. His family history seemed unimportant, and apart from infectious fevers in childhood he had never been ill before. For the previous five years he had been engaged in working with nitrous oxide; one of his duties was to test the pressure of nitrous oxide in some 300 tanks every day by opening the tanks; in this way he inhaled the gas in considerable amounts. For about three days one month before the onset of his symptoms the tanks contained nitric oxide; at this time the patient felt dizzy and nauseated each evening. A month later he suddenly felt ill with headache and dyspnoea on exertion. He became increasingly weak and pale. Ten weeks before admission he was admitted to another hospital where he was found to be extremely anaemic, and was treated with four transfusions and a course of liver extract parenterally. He also was given X-ray treatment over an enlarged spleen. He had no haemorrhages and no symptoms except those of anaemia.

*Physical examination.* Temperature 99.0° F., pulse 118. Well developed and well nourished. Extreme pallor and considerable racial pigmentation. Conjunctivae slightly jaundiced, several haemorrhages in right fundus. Tongue normal. Haemic murmur. Liver and spleen felt at costal margin. A few lymphatic glands the size of peas felt in both cervical chains. No other lymphatic glands palpable. No neurological abnormality.

*Laboratory investigations.* Blood count on admission, red cells 900,000 per c.mm., haemoglobin 21 per cent., colour index 1.17, mean corpuscular volume 106 cub.  $\mu$ , platelets 308,000 per c.mm., white cells 1,000 per c.mm.; differential count, polymorphonuclear leucocytes 75 per cent., lymphocytes 20 per cent., monocytes 4 per cent., basophils 1 per cent. A few myelocytes were seen in some films. Autoagglutination occurred when blood was allowed to cool. Radiograph of chest, no abnormality seen.

*Biopsy of sternal marrow.* Bone spicules appear normal. Marrow hypercellular with little fat. Numerous haemocytoblasts, primary erythroblasts, and normoblasts, also myelocytes and polymorphonuclears. In some areas there are numerous immature basophil cells and eosinophil myelocytes. Some areas with spindle fibroblasts and eosinophil fibrous tissue. Some haemosiderosis, numerous megakaryocytes.

*Further course.* The patient was admitted four times in 10 months. During the whole time that he was under observation he had considerable irregular fever (to 102° or 103° F.) and frequent profuse attacks of perspiration. During the first month after admission the spleen enlarged until its lower border was at the level of the umbilicus. In spite of numerous treatments and transfusions the patient's condition slowly deteriorated, except for one slight but definite temporary improvement in clinical condition and blood picture five months after his first admission. His general condition was little changed except that he was troubled with nausea and vomiting. There were no retinal or skin haemorrhages; there was no enlargement of lymphatic glands; the liver was easily palpable below the costal margin, and the spleen was easily palpable 8.0 cm. below the costal margin. The blood count was, red cells 700,000 per c.mm., haemoglobin 13 per cent., colour index 1.0, white cells 3,100 per c.mm.; differential count, polymorphonuclear leucocytes 29 per cent., lymphocytes 47 per cent., monocytes 24 per cent. Severe vomiting continued and the patient died three days after admission, 12 months after his first symptom.

*Summary of autopsy findings.* Body that of a well developed and well nourished man. Diffuse brown, probably racial, pigmentation. Sclerae and subcutaneous fat distinctly yellow. Several mesenteric lymphatic glands slightly enlarged with opaque mottled-white cut surface and elastic consistency. No enlargement of mediastinal lymphatic glands, one hilum gland shows opaque white area 0.4 cm. diam. Oedema of lungs. Spleen (610 gm.) greatly enlarged, cut surface shows irregular mottled grey-white depressed areas (0.1 to 0.5 cm. in diam.) on a uniform purple-red background. There are also areas apparently of fibrosis. Liver (2,300 gm.) greatly enlarged, and golden-brown in colour, with portal areas sharply demarcated by grey-white areas of fibrosis. Kidneys enlarged (250 and 260 gm.), pattern obliterated on firm opaque yellowish-white surface. Pancreas appears normal except for uniform golden-brown colour. Femoral, sternal, and vertebral marrow dense in consistency with areas of firm greyish-red or greyish-yellow tissue separated by deep red marrow.

*Summary of microscopical findings.* All sections densely hypercellular. Marrow is of the partly mature cellular type with numerous polymorphonuclears and normoblasts. Normal maturation is therefore present and the picture does not suggest leukaemia. Numerous megakaryocytes. Occasional small areas with fibroblasts and some fibrous tissue. Spleen, extramedullary haemopoiesis and haemosiderosis, megakaryocytes in one section. Lymph glands, extramedullary haemopoiesis and haemosiderosis. Liver, severe central fatty degeneration, haemosiderosis, and some extramedullary haemopoiesis at portal areas.

### 7. Remissions in Refractory Anaemia

Remissions, apparently spontaneous and sometimes dramatic, occur in refractory anaemia, and this is not well known. This fact suggests that the

outlook for the discovery of a successful form of therapy is not as remote as it otherwise appears.

In eight of our patients there was a history of a partial and temporary remission early in the course of the disease and before the patients came under our observation. In six of these improvement appeared to follow parenteral liver extract therapy, in five of these six the marrow at a subsequent biopsy was of the partly mature cellular type, and in the sixth the absence of severe leucopenia and thrombocytopenia suggested a marrow of the same type. Though temporary, these ameliorations appeared definite with increases of haemoglobin of from 25 to 40 per cent. All of the six patients subsequently failed to respond favourably to any form of therapy, including liver extract, and eventually died; it seems likely that these cases represent the condition described as 'achrestic anaemia' by Israëls and Wilkinson (1936).

Excluding these examples of temporary improvement, remissions occurred in 15 cases in this series. In four instances (including Cases 28 and 29) the patients had not consulted a doctor, but were found to have a mild anaemia, moderately severe leucopenia, and thrombocytopenia at a routine examination of workers exposed to benzol in a printing shop. They were removed from contact with benzol, and in each case a gradual remission occurred. In eight of the remaining 11 cases there was a history of exposure to some kind of potentially toxic substance, but in three there was no such history. It seems therefore that remissions are commoner in patients whose disease can be attributed to exposure to an exogenous toxin.

Biopsy examinations of sternal marrow were made in 12 of the 15 cases; in six the marrow was of the partly mature cellular type, in five of the hypocellular type, and in one of the immature cellular type. Of the five cases with marrow of the hypocellular type, the reduction in cellularity was distinct but moderate in degree, with intervening areas of active haemopoiesis in three; it was severe in two (Cases 17 and 24), in one of which (Case 17) the sternal marrow was the most severely hypocellular example seen in the whole series of biopsy and autopsy sections of sternal marrow. Remissions therefore most commonly occur in patients with cellular marrow of the partly mature type, but may occur even in the presence of severe hypoplasia of the sternal marrow.

The course of the remissions presents features of considerable interest. In nine patients, eight of whom had recently been removed from exposure to a definite exogenous toxin, the remissions occurred fairly rapidly, and the haemoglobin returned to within the normal range in from two to seven months. Biopsies had been performed on eight of these patients and none of them had a severely hypocellular marrow. The remaining patient (Case 22), in whom there was no history of exposure to toxic substances, was one in whom three remissions had occurred in the course of four years. The whole picture of this patient, including the course of the remissions, differed from that of the others and it will therefore be discussed separately. In four

further patients the course of the remission was very much slower, normal or nearly normal haemoglobin figures being reached in from eight months to two years. In two of these the marrow was hypocellular (Cases 17 and 18), in one it was of the partly mature type (Case 19), and in one no biopsy had been performed (Case 20). In the remaining two patients details of the blood counts during the course of the remission are not available. In every case but one, of those in which details were available, the haemoglobin percentage increased more rapidly than the number of red cells, so that the colour index and mean corpuscular volume increased as the remissions occurred. Colour indices as high as 1.5 and mean corpuscular volumes as high as 130 cub.  $\mu$ , were seen in a few instances (e.g. Case 19, Fig. 1).

The first signs of a commencing remission was that transfusions had a greater effect on the blood count than previously, so that to maintain a given average count it was necessary to transfuse at increasingly longer intervals. This was well demonstrated by Case 17. At the same time or a little later there was often an increase in the colour index, an increasing mean corpuscular volume, and an increase in the white cell count. Once a remission began, the haemoglobin increased at first relatively rapidly in the majority of cases to a certain level and then more slowly to within a normal range. In a few of the milder cases the red cell count became normal almost as soon as the haemoglobin; in the majority the red cell count remained significantly below normal for a considerable time after the haemoglobin was normal. In a few instances this state of affairs persisted as long as the patients were under observation, as for instance in Case 19 (Fig. 1) where the haemoglobin remained at about the lower limits of normal, the red cell count was distinctly below normal, and there was slight macrocytosis for a period of some 20 months.

We can do no more than guess at the significance of this rising colour index during remissions and the persistence of macrocytosis and a low red cell count. It is worth noticing that the blood picture in these patients, as the remission proceeded, came to resemble exactly that seen in certain liver diseases. The evidence of liver damage *post mortem* has already been discussed, and in a later section biochemical evidence of liver dysfunction during life will be presented. It is possible that in all but the mildest cases, where a remission occurs, the patients are left with some residual liver damage, which is responsible for the persistent abnormality in their blood picture. If this is the case, it is possible that it is the degree of liver dysfunction, at present notoriously difficult to estimate, which determines whether a patient goes into a remission or not.

The course of the remissions in a case (Case 22) so far excluded from this discussion is shown in Fig. 2. This was a patient with marrow of the partly mature type in several biopsy specimens, and with an enlarged spleen which increased in size in relapse and diminished in each remission. In this case no increase in the colour index and red cell size was seen during remissions. The first remission occurred dramatically after the patient had been severely

anaemic for 15 months, and coincided with an attack of obstructive, apparently catarrhal, jaundice. Treatment with cholesterol, beef bile, and bile pigment by mouth during the subsequent relapse was without effect, as was a transfusion from a patient with obstructive jaundice. The second remission, which was almost as dramatic, occurred some two years later when the patient was being treated with ferrous sulphate by mouth. The third occurred more slowly, about 18 months after the second, at a time when no treatment was being given. The question of the relationship of the treatment given to the remissions in these cases is a difficult one. Looking at individual cases it would be easy to conclude that the treatment given was effective, e.g. ferrous sulphate in Case 22, vitamin B<sub>1</sub> in Case 20, and ventriculin and raw liver in Case 18. In view of the variety of treatment which appeared effective in different cases, and of the fact that each of these treatments was found quite ineffective in other cases, it seems only possible to conclude that the remissions were spontaneous and had no relation to the treatment given.

### *Case Reports*

Refractory anaemia with severely hypocellular marrow, followed by remission.

*Case 17.* An American photostat operator, 19 years old, first complained of nose bleeding four months before the time of his admission to the Rockefeller Institute Hospital. His previous history and family history appeared unimportant. The patient left school at 18 years, and eight months before admission became a photostat operator exposed to chemicals, including rhodol and hydroquinone. Four months before admission he felt unduly tired and appeared pale. Nose bleeds of increasing severity occurred, and later purpura. He was found to have severe anaemia and leucopenia, for which he was given 10 transfusions.

*Physical examination.* Temperature 98.2° F., pulse 120. Well developed and well nourished. Very pale. A few petechiae and retinal haemorrhages. Tongue normal, blood clot in left nostril and in pharynx. Chest and heart normal. No enlargement of liver or spleen. Very slight enlargement of a few posterior cervical and inguinal lymphatic glands. No neurological abnormality.

*Laboratory investigations.* Blood count on admission, red cells 2,100,000 per c.mm., haemoglobin 62 per cent., colour index 1.44, mean corpuscular volume 99 cub.  $\mu$ , reticulocytes 3.6 per cent., platelets 166,000 c.mm., white cells 3,500 per c.mm.; differential count, polymorphonuclear leucocytes 23 per cent., lymphocytes 70 per cent., monocytes 7 per cent.; films, slight anisocytosis, no poikilocytosis, many macrocytes. Icterus index 4. Test meal, free hydrochloric acid present after histamine. Haemolysis of red cells in saline began at 0.44 per cent. and was complete at 0.36 per cent. (control 0.46 to 0.34 per cent.). Blood ascorbic acid 1.0 mg. per 100 c.c. Average daily urobilinogen output, stools 156 mg., urine 0.9 mg. Radiographs of chest and long bones showed no abnormality.

*Biopsy of sternal marrow.* Practically the whole section consists of fat with a few vessels containing red cells. One group of four or five small round cells. This was the only completely 'aplastic' marrow seen in the series.

*Further course.* The patient remained in hospital for ten months. His

usual temperature was 99° F., with an occasional rise to 101° F., and sharp rises, often with a rigor, after transfusions. During the first month haemorrhages into both retinae in the macular regions produced partial blindness. Shortly after admission, while under treatment with ultraviolet radiation, the patient developed a skin rash diagnosed as seborrhoeic dermatitis. At the same time his red cell count decreased to 570,000 per c.mm., and the reticulocyte count rose to 7.7 per cent. For the first four months he needed a transfusion every week or 10 days, thereafter less frequently. The red cell count remained about 1,000,000 per c.mm. The platelets fell to 46,000 per c.mm. Six months after admission the patient developed increased intra-ocular tension with slight exophthalmos, and became almost blind. As an operation was considered inadvisable, he was treated with eserine and pilocarpine. In the last four months of his time in hospital there was some symptomatic improvement, transfusions were needed less frequently, and his blood count rose slightly. Eight months after admission his blood count was, red cells 1,700,000 per c.mm., haemoglobin 45 per cent., colour index 1.3, white cells 1,850 per c.mm. He was treated with citron, liver extract, vitamin C, vitamin A, acidophilus milk, betaxan, and 16 transfusions. He was discharged after 10 months. In the next four months he returned for three further transfusions. After this his red cell count remained stationary and his white cell count increased slowly without more transfusions. Nine months after discharge his blood count was, red cells 1,700,000 per c.mm., haemoglobin 42 per cent., colour index 1.2, white cells 5,000 per c.mm.; differential count, polymorphonuclears 22 per cent., lymphocytes 73 per cent., eosinophils 2 per cent., neutrophil myelocytes 2 per cent., normoblasts 1 per cent., and 19 months after discharge it was red cells 3,200,000, haemoglobin 70 per cent., and white cells 6,200 per c.mm.

Refractory anaemia with hypocellular marrow, followed by remission.

*Case 18.* A married Italian woman, who had been in America 10 years, first complained of pallor at the age of 24 years, eight months before the time of her admission to the Rockefeller Institute Hospital. Her family history appeared unimportant, and her diet had been a good one. For many years she had had recurrent attacks of acute bronchitis. From the age of 18 years onwards she had had mental symptoms which had been diagnosed as recurrent paranoid episodes. No history of exposure to toxic chemicals was obtained. Her menstruation had been normal and regular. Nine months before admission, after an attack of bronchitis, she complained of pallor and sore throats. Her tonsils were removed without undue haemorrhage. Six months before admission she had had some menorrhagia, and four months before admission she was found to be anaemic. Menstruation had ceased and she was found to be pregnant. Iron therapy and 34 injections of liver extract produced no improvement; a therapeutic abortion was therefore performed, and she was given five blood transfusions. She then went home, but her symptoms continued and she had occasional epistaxis.

*Physical examination.* Temperature 99.8° F., pulse 108. Well developed and well nourished woman. Pale, with distinctly yellowish skin and conjunctivae. No retinal or skin haemorrhages. Tongue normal. Chest normal. Haemic murmur. Liver and spleen just felt. No enlargement of lymphatic glands. No neurological abnormality.

*Laboratory investigations.* Blood count on admission, red cells 1,000,000 per c.mm., haemoglobin 24 per cent., colour index 1.2, mean corpuscular volume 94 cub.  $\mu$ , platelets 44,000 per c.mm., white cells 2,500 per c.mm.;

differential count, polymorphonuclear leucocytes 35 per cent., lymphocytes 63 per cent., eosinophils 2 per cent. Haemolysis of red cells in saline begins at 0.3 per cent. and is complete at 0.4 per cent. (control 0.3 to 0.4 per cent.). Test meal, free hydrochloric acid present. Barium enema, no abnormality discovered.

*Biopsy of sternal marrow.* (Three months before admission; section kindly sent from Fifth Avenue Hospital.) Sections show patchy hypocellular marrow with areas of almost normal cellularity, and areas of fat with scanty groups and lines of haemopoietic cells separating fat cells. There are very numerous primary erythroblasts, and numerous normoblasts. Relatively fewer myelocytes, mostly eosinophil, and polymorphonuclear leucocytes. Very occasional haemocytoblasts, no megakaryocytes seen. The marrow was therefore hypoplastic and predominantly erythropoietic.

*Further course.* The patient was in hospital for eight months, with one small interval. During the whole period she had slight fever, to about 100° F. She had at one time slight bleeding from the gums, and developed one haemorrhage in the left retina. She was treated with large doses of liver extract intravenously, and with reduced iron, arsenic, haemoglobin scales, autoclaved vegex, a vitamin digest containing beef intestine, yeast, and vitamins A, B, C, and D, and whole raw liver by mouth, all without evident benefit. She also received 22 transfusions. On discharge her blood count was, red cells 1,900,000 per c.mm., haemoglobin 46 per cent., white cells 1,700 per c.mm. Three months after discharge, during which time the patient had been taking ventriculin and whole raw liver by mouth, her blood count was, red cells 2,300,000 per c.mm., haemoglobin 65 per cent., colour index 1.4, mean corpuscular volume 116 cub.  $\mu$ , white cells 6,700 per c.mm. Thereafter the patient attended erratically. Sixteen months later her blood count was, red cells 3,900,000 per c.mm., haemoglobin 92 per cent., colour index 1.2, mean corpuscular volume 96 cub.  $\mu$ , white cells 8,100 per c.mm. Two years after discharge, and nearly three years after her first symptoms, her blood count was, red cells 3,200,000 per c.mm., haemoglobin 93 per cent., colour index 1.4, white cells 5,300 per c.mm. It was found impossible to trace the patient after this date, but it is known that she was alive and active 4½ years after discharge.

Refractory anaemia after exposure to benzol in a patient possibly sensitized by previous arsphenamine therapy. Partly mature hypercellular marrow. Remission.

*Case 19.* A beauty parlour operator of Russian extraction who had been in America 10 years first complained of undue lassitude at the age of 35 years, three months before the time of her admission to the Rockefeller Institute Hospital. Her family history appeared unimportant. Menstruation had been normal and regular, and her diet had been good. Fourteen years before admission her husband had had a luetic infection, and her Wassermann reaction had been found to be positive. For the next 13 years she had taken a course of bismuth and salvarsan injections almost every year, although her Wassermann reaction after the first occasion had always been negative. Six or seven months before admission she began to use at her work a rapid hair cleaner containing 30 per cent. of benzol. She used one pint of this preparation six or seven times a week. About three months before admission she felt unduly tired, and suffered on two occasions from nausea, vomiting, and faintness, when she washed her own hair with it. She was found to be anaemic, and developed subcutaneous ecchymoses and

uterine bleeding. Five weeks before admission her blood count was, red cells 800,000 per c.mm., haemoglobin 15 per cent., colour index 1.0, reticulocytes 7.0 per cent., platelets 29,000 per c.mm., white cells 4,300 per c.mm. She was treated with iron and liver extract, and given five transfusions.

*Physical examination.* Temperature 99.6° F., pulse 108. Well developed and slightly obese. Petechiae on arms and legs. No retinal haemorrhages. Tongue normal. Haemic murmur. No enlargement of liver, spleen, or lymphatic glands. No neurological abnormality.

*Laboratory investigations.* Blood count on admissions, red cells 2,200,000 per c.mm., haemoglobin 49 per cent., colour index 1.17, mean corpuscular

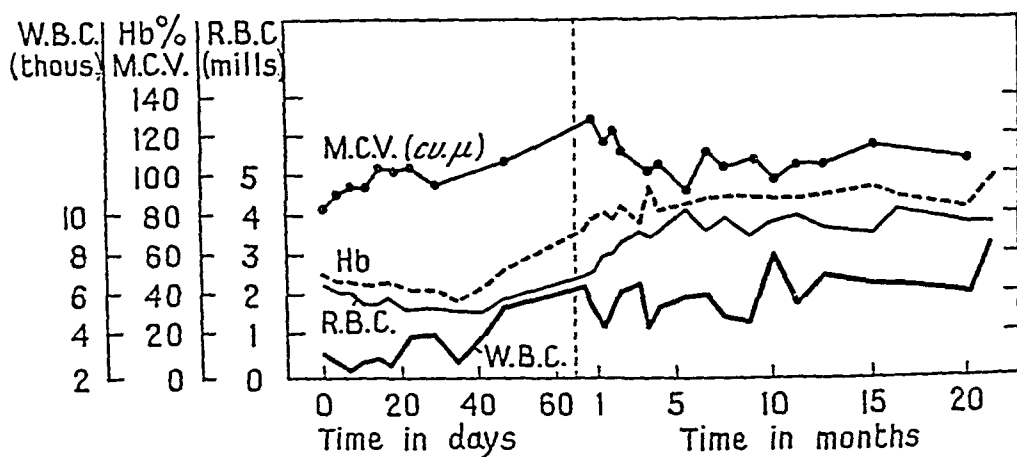


FIG. 1. Case 19. The figure illustrates the usual course of an incomplete spontaneous remission in refractory anaemia. The beginning of the remission was indicated by an increase in the white cell count, in the colour index, and in the mean corpuscular volume. The figures for red cells and haemoglobin did not reach normal. A mild macrocytic anaemia, closely resembling that seen in diseases of the liver, persisted as long as the patient remained under observation.

volume 84 cub.  $\mu$ , platelets 20,000 per c.mm., white cells 2,950 per c.mm.; differential count, polymorphonuclear leucocytes 49 per cent., lymphocytes 48 per cent., monocytes 2 per cent., myelocytes 1 per cent., occasional nucleated red cells seen. Icterus index 7. Bleeding time 15 min.

*Biopsy of sternal marrow.* Section contains large areas of haemorrhage with islands of haemopoietic tissue, and the latter appear hypercellular. Most areas consist mainly of haemocytoblasts, primary erythroblasts, and normoblasts; other areas contain numerous myelocytes, many being eosinophil, and a few polymorphonuclear leucocytes. No megakaryocytes seen. The marrow is therefore a partly mature, predominantly erythropoietic one.

*Further course.* The patient had slight fever, to about 100° F. She continued to have a few petechiae on the limbs for several weeks, and a small ulcer, which appeared on the left tonsil, healed uneventfully. She was treated in turn with ventriculin, vegex, and raw liver. Her blood count on discharge, seven weeks after admission, was, red cells 1,900,000 per c.mm., haemoglobin 53 per cent., colour index 1.4, mean corpuscular volume 108 cub.  $\mu$ , white cells 5,700 per c.mm. Five weeks later it was, red cells 3,700,000 per c.mm., haemoglobin 91 per cent., white cells 7,000 per c.mm. Seven months after discharge and 10 months after her first symptom it was, red cells 3,800,000 per c.mm., haemoglobin 81 per cent., colour index 1.08, white cells 7,200 per c.mm. The patient was then feeling quite well and was



back at work, being careful to avoid further exposure to benzol, but her blood count has remained practically unaltered up to the time of writing.

Refractory anaemia, possibly associated with a poor diet and the taking of drugs of the pyramidon-barbiturate group, with remission.

*Case 20.* A single American teacher first complained of pallor and weakness at the age of 42 years, one year before the time of her admission to the Rockefeller Institute Hospital. Her family history appeared unimportant, and apart from childhood infections and an appendicectomy she was well up till the age of 34 years. She then developed constipation, feelings of nervousness and weakness, and a tendency to blush easily. From that time onwards she took luminal, medinal, and phenolphthalein, in considerable quantities, and also at times calomel, atophan, phenacetin, and tablets containing aspirin and amidopyrine. Her diet had been a poor one, but she had not lost weight. Her menstruation was regular and normal. In the six months before admission, she was given two transfusions. Slight bleeding occurred from the gums.

*Physical examination.* Temperature 99.2° F., pulse 88. Well developed and well nourished. Tongue normal. No skin or retinal haemorrhages. Haemic murmur. No enlargement of liver, spleen, or lymphatic glands. No neurological abnormality.

*Laboratory investigations.* Blood count on admission, red cells 1,200,000 per c.mm., haemoglobin 32 per cent., colour index 1.3, mean corpuscular volume 132 cub.  $\mu$ , reticulocytes 3 per cent., platelets 48,000 per c.mm., white cells 1,750 per c.mm.; differential count, polymorphonuclear leucocytes 26 per cent., lymphocytes 64 per cent., monocytes 10 per cent., one nucleated red cell seen in counting 100 white cells. Icterus index 5 and 10 units on different occasions. Plasma bilirubin 1.5 mg. per 100 c.c. Average daily urobilinogen excretion, stools 189 mg., urine trace.

*Further course.* This patient was in the hospital for five months. She was usually afebrile with occasional fever to 99.5° F. Severe anaemia persisted and her blood count on discharge was, red cells 1,300,000 per c.mm., haemoglobin 31 per cent., white cells 1,650 per c.mm. All medication with pyramidon or barbiturates was discontinued. She was treated with liver extract, nicotinic acid, and indol-acetic acid at different times, without evident benefit. She was also given two transfusions. After discharge the patient was treated with vitamin B<sub>1</sub> by injection thrice weekly. Her general condition and blood count slowly improved. Eleven months after discharge and one year after the last transfusion her blood count was, red cells 3,600,000 per c.mm., haemoglobin 79 per cent., colour index 1.1, white cells 5,650 per c.mm.; differential count, polymorphonuclear leucocytes 50 per cent., eosinophils 4 per cent., lymphocytes 38 per cent., monocytes 2 per cent. Up till the time of writing, six months later, she has remained clinically well and active, but her blood count has remained at almost exactly the same level.

Refractory anaemia with hypercellular partly mature marrow, after exposure to benzol. Evidence of haemolysis. Complete remission.

*Case 21.* An American chemist, 23 years old first complained of pallor and poor appetite three weeks before the time of his admission to the Rockefeller Institute Hospital. His family and previous history appeared unimportant, and his diet had been good. Up till 18 months before admission he had worked in a filling station. Since that time he had worked

in a laboratory using benzoic acid and benzol as a solvent for about two weeks each month. He last used benzol six weeks before admission. Three weeks before admission he consulted a doctor and was found to have fever and anaemia with a haemoglobin of 50 per cent. He also complained of swollen painful gums. In view of the finding of an enlarged spleen and the presence of immature white cells in blood films a diagnosis of leukaemia was made.

*Physical examination.* Temperature 100.4° F., pulse 120. Well developed and well nourished. Pale and sallow, but not jaundiced. Few old ecchymoses on thighs, no skin or retinal haemorrhages. Tongue normal, gums oedematous and swollen. Chest and heart normal. No enlargement of liver or lymphatic glands. Spleen just palpable. No neurological abnormality.

*Laboratory investigations.* Blood count on admission, red cells 2,200,000 per c.mm., haemoglobin 51 per cent., colour index 1.16, mean corpuscular volume 101 cub.  $\mu$ , reticulocytes 12 per cent., platelets 120,000 per c.mm., white cells 2,400 per c.mm.; differential count, polymorphonuclear leucocytes 16 per cent., lymphocytes 70 per cent., monocytes 14 per cent. Films, polymorphonuclears appear abnormal with poor granulation in the cytoplasm. Myelocytes (2 or 3 per cent.) were seen in some films. Icterus index 15. Average daily urobilinogen output, urine 1.5 mg., stools 570 mg. Haemolysis of red cells in saline begins at 0.42 per cent., and is complete at 0.34 per cent. (control 0.44 to 0.34 per cent.).

*Biopsy of sternal marrow.* Sections somewhat fragmented. There are sheets of densely cellular marrow with almost no fat. In some of them there are a few haemocytoblasts, numerous primary erythroblasts, and moderate numbers of normoblasts; in others there are few haemocytoblasts and numerous myelocytes with very few polymorphonuclear leucocytes. Few megakaryocytes. The marrow is a hypercellular partly mature one with erythropoiesis more active than leucopoiesis.

*Further course.* During his two weeks in hospital the patient had moderate irregular fever and his condition remained unchanged. After discharge he was treated with ascorbic acid, 125 mg. daily, and Valentine's liver extract, 90 c.c. daily by mouth. Two months after discharge his blood count was, red cells 4,000,000 per c.mm., haemoglobin 90 per cent., white cells 7,000 per c.mm., and seven months after discharge it was, red cells 5,600,000 per c.mm., haemoglobin 112 per cent., and white cells 5,900 per c.mm. The patient has avoided further exposure to benzol and has remained well; his blood count, followed for a year after discharge, remained normal.

Refractory anaemia with hypercellular partly mature marrow. Prolonged course with three remissions, one apparently after an attack of obstructive jaundice.

*Case 22.* An American Jewish schoolboy first complained of spontaneous epistaxis at the age of 17 years, 18 months before the time of his admission to the Rockefeller Institute Hospital. The family history and previous history appeared unimportant, and the patient had eaten a good diet. Epistaxis recurred at intervals, and after a few months the patient was noticeably pale. His haemoglobin was found to be 23 per cent. Three months later he was admitted to Mount Sinai Hospital. He was found to have signs of heart disease, and his spleen was palpable 4.0 cm. below the costal margin. There were a few retinal haemorrhages. His blood count was, red cells 1,100,000 per c.mm., haemoglobin 21 per cent., white cells 6,600 per c.mm.; differential count, normal; no reticulocytes seen. A biopsy of

the sternal marrow and of a lymphatic gland was performed, but no final diagnosis could be made. He was given a variety of treatments, including large amounts of liver extract, iron, and stomach preparations, and in all 12 transfusions from which he obtained temporary benefit only. During this time he continued to have severe anaemia and recurrent fever up to 104° F.

*Physical examination.* Temperature 98.2° F., pulse 100. Well developed and well nourished. Very pale, red-headed boy with slightly jaundiced conjunctivae. Tongue normal. No skin or retinal haemorrhages; dried blood in left nostril. Small lymphatic glands palpable in neck and in both axillae

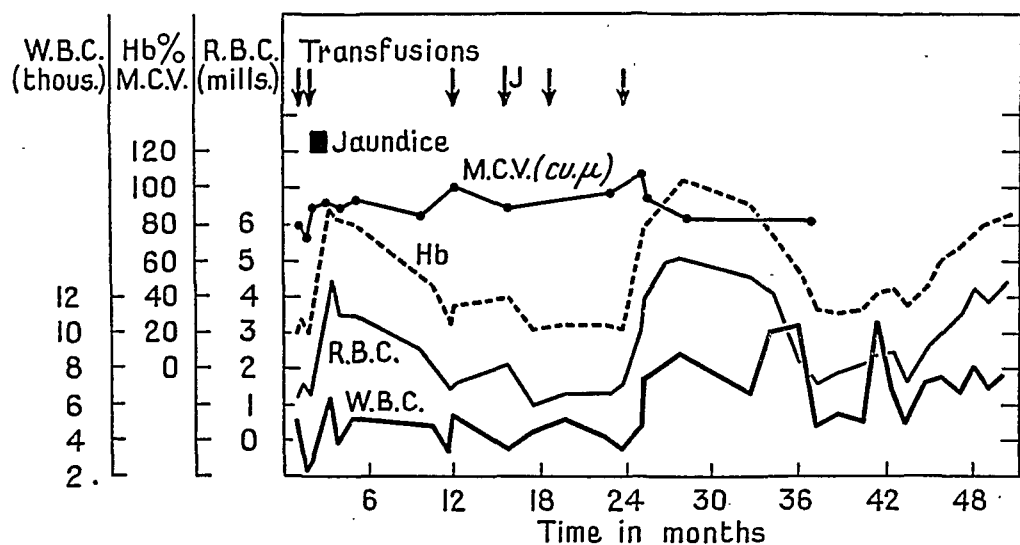


FIG. 2. Case 22. The figure shows the course of an exceptional case of refractory anaemia, in which three remissions occurred in four years. The first and most dramatic coincided with an attack of catarrhal jaundice. In the subsequent relapse a transfusion from a patient with obstructive jaundice (arrow with J in chart) was without beneficial effect. An unusual feature of this case was the absence of a significant increase in the colour index and the mean corpuscular volume during the course of the remissions.

and groins. Heart, apex beat palpable outside nipple line, with loud systolic murmur heard all over praecordium and loudest over pulmonary area. Blood pressure 158/40. Spleen palpable 4.5 cm. below costal margin; liver not felt. No neurological abnormality. Slight pitting oedema of feet.

*Laboratory investigations.* Blood count on admission, red cells 1,200,000 per c.mm., haemoglobin 20 per cent., colour index 0.84, mean corpuscular volume 78 cub.  $\mu$ , reticulocytes 0.4 per cent., platelets 300,000 per cub. mm., white cells 5,050 per c.mm.; differential count, polymorphonuclear leucocytes 41 per cent., lymphocytes 49 per cent., monocytes 5 per cent., eosinophils 3 per cent., basophils 1 per cent., myelocytes 1 per cent. Wassermann reaction negative. Icterus index 8. Haemolysis of red cells in saline begins at 0.40 per cent. and is complete at 0.30 per cent. (control 0.4 to 0.38 per cent.). Test meal, free hydrochloric acid present. Plasma bilirubin 0.7 mg. per 100 c.c. Urea clearance, first sample 94 per cent. of normal, second sample 100 per cent. of normal. Average daily urobilinogen excretion, urine 3.9 mg., stools 178 mg.

*Biopsy of sternal marrow.* Section from Mount Sinai Hospital made 10 months before time of admission to Rockefeller Institute Hospital. Section shows normal bone spicules separated by moderately densely hypercellular

marrow with no fat cells; slight haemosiderosis. There are haemocyto blasts, sometimes in groups, some primary erythroblasts, and conspicuous groups of basophil normoblasts, often of large size; some myelocytes, numerous polymorphonuclear leucocytes, and numerous megakaryocytes. The picture is that of a partly mature marrow with normal leucopoiesis and relatively increased erythropoiesis, the latter marked by an increase in haemocyto blasts, primary erythroblasts, and basophil normoblasts.

Rockefeller Institute Hospital section. Marrow still hypercellular without fat. Erythropoiesis conspicuous with numerous haemocyto blasts and erythroblasts, and relatively fewer normoblasts. Myelocytes, often eosinophil, in moderate numbers and few polymorphonuclears. Megakaryocytes about normal in number. The marrow is therefore similar to that in the previous biopsy, with a relative increase in immature cell forms.

*Further course.* This patient was admitted five times in the next four years (Fig. 2). During each admission he had periodic bouts of fever. At times these took the form of sudden rises to  $102^{\circ}$  or  $103^{\circ}$  F. for periods of 24 to 72 hours, sometimes with a rigor; at another time there was a typically Pel-Ebstein type of fever for a period of several months. One month after his first admission the patient had an attack of obstructive jaundice, apparently catarrhal in nature, lasting about four weeks. His blood count, which had remained almost constant at very low levels for the previous 15 months, immediately began to rise and, as shown in Fig. 2, his haemoglobin increased from 32 to 90 per cent. in  $5\frac{1}{2}$  weeks. At the same time his general condition improved and the spleen and lymphatic glands decreased in size, though the spleen remained palpable. The patient's subsequent course can be followed in the Figure. His blood count decreased slowly over the next seven months. A period of intramuscular liver extract therapy produced no improvement. As the remission in his disease appeared to have been associated with his attack of jaundice, he was treated in turn with 45 c.c. daily by mouth of a solution of 1 per cent. cholesterol in olive oil, with bile from 25 to 100 c.c. daily by stomach tube, and with beef bile pigment 3 gm. daily by mouth, without any significant change in his blood count. As his blood count fell, his spleen increased to its previous size. Twenty-one months after his first admission the patient was readmitted for further investigation. On physical examination at this time the cardiac signs were unchanged, the lymphatic glands in the neck, axillae, and groins were slightly enlarged, the liver was easily palpable and the spleen was enlarged almost to the umbilicus. The blood count was, red cells 1,900,000 per c.mm., haemoglobin 28 per cent., reticulocytes 1 per cent., platelets 300,000 per c.mm., white cells 3,300 per c.mm.; differential count, polymorphonuclear leucocytes 53 per cent., eosinophils 2 per cent., lymphocytes 38 per cent., monocytes 7 per cent.; films showed anisocytosis and a little poikilocytosis. The plasma bilirubin was 0.3 mg. per 100 c.c., and the average daily urobilinogen output was, stools 175 mg., urine 17.0 mg. and 28 mg. in two separate three-day periods. The increased excretion of urobilinogen in the urine suggested impaired hepatic function; X-ray examination with an opaque meal gave no evidence of oesophageal varices.

Biopsy of an axillary lymphatic gland and a further bone marrow biopsy were performed. Sections from the lymphatic gland showed extramedullary haemopoiesis, but no evidence of Hodgkin's disease. The specimen of marrow removed at this time was densely hypercellular, with numerous haemocyto blasts often in groups and relatively fewer erythroblasts and normoblasts; numerous myelocytes, often eosinophil, and very few polymorphonuclear

leucocytes. No megakaryocytes seen. The marrow was thus similar in type to that in previous biopsies, with a relative predominance of erythropoiesis, but it had become progressively more immature. At this time the patient had a swinging fever with peaks recurring weekly and intervening periods of subnormal temperature. This began about four months before his second admission and was still present when he was discharged three months later. He was discharged unimproved on ferrous sulphate 0.6 gm. twice daily. Almost immediately he had a second remission as shown in Fig. 2, and his haemoglobin increased in four months from 22 to 104 per cent. It seems unlikely that the iron medication was responsible for this increase. At the same time his fever practically disappeared, and his spleen receded to within three fingers breadth of the costal margin. Slight enlargement of the lymphatic glands persisted. His blood count decreased slowly over the next seven months, and then more rapidly, and was at low levels for a further six months. Forty-four months after his first admission, and about five years after his first symptoms a third remission began, apparently quite spontaneously, and has continued up till the time of his last visit.

### *Summary*

A series of 58 cases of anaemia, which proved resistant to any form of treatment except blood transfusions, was divided according to the histological appearances in sections of the bone marrow into four groups.

1. Refractory anaemia with partly mature cellular marrow, or Pseudo-aplastic anaemia (31 cases). In this group the bone marrow differed least from the normal, symptoms were relatively mild, the illness was sometimes of long duration, and spontaneous remissions were not uncommon. Leucopenia and thrombocytopenia were inconspicuous or absent, and haemorrhages and infections occurred infrequently. Pigmentation of the skin was seen as a complication, and less frequently haemochromatosis. In some cases this type of anaemia appeared to be a temporary phase in the development of other disorders of the haemopoietic system.

2. Refractory anaemia with hypoplastic marrow, or Aplastic anaemia (11 cases). In this group, as compared with the previous one, the duration of the illness was usually shorter, symptoms, especially haemorrhage, were more severe and spontaneous remissions occurred infrequently. Leucopenia and thrombocytopenia were present in some degree in all cases.

3. Refractory anaemia with immature cellular marrow, or Chronic granulocytopenia (12 cases). In this group the bone marrow contained very large numbers of erythroblasts, and on superficial examination presented an appearance not unlike that of lymphatic leukaemia. There was usually no more than moderate anaemia, but severe and progressive granulocytopenia, often with considerable numbers of immature white cells in the peripheral blood stream. The average duration of the illness was relatively short, and the most prominent clinical feature was the occurrence of areas of necrosis and infection, particularly in the neighbourhood of the mouth and anus. A remission occurred in one case only.

4. Refractory anaemia with fibrosis, sclerosis and giant cell hyperplasia of the marrow, or Myelosclerosis (4 cases). In this group the duration of the

illness was relatively short and the outcome uniformly fatal. The most prominent clinical feature was a progressive and considerable enlargement of the spleen and liver.

The onset of a remission in refractory anaemia is usually indicated by an increase in the white cell count, the colour index, and the mean corpuscular volume. Remissions may be complete or incomplete. When they are incomplete the blood picture comes to resemble closely that seen in the mild macrocytic anaemia of liver disease.

Acknowledgements and references will be found at the end of Part II.

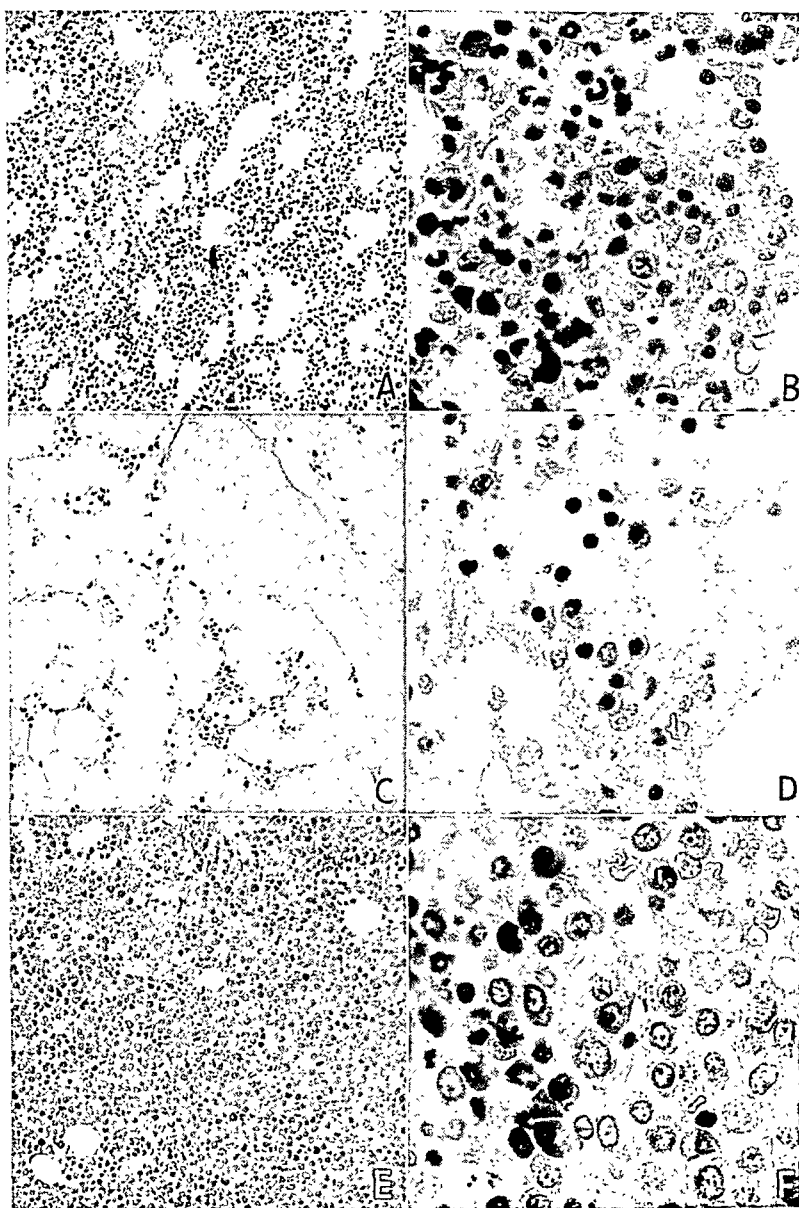


FIG. 3. Sternal marrow of normal cellularity. Magnification A  $\times 125$ , B  $\times 500$ . Sternal marrow in refractory anaemia with hypocellular marrow. Magnification C  $\times 125$ , D  $\times 500$ . Sternal marrow in refractory anaemia with immature cellular marrow. Magnification E  $\times 125$ , F  $\times 500$ .

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usual temperature was 99° F., with an occasional rise to 101° F., and sharp rises, often with a rigor, after transfusions. During the first month haemorrhages into both retinae in the macular regions produced partial blindness. Shortly after admission, while under treatment with ultraviolet radiation, the patient developed a skin rash diagnosed as seborrhoeic dermatitis. At the same time his red cell count decreased to 570,000 per c.mm., and the reticulocyte count rose to 7.7 per cent. For the first four months he needed a transfusion every week or 10 days, thereafter less frequently. The red cell count remained about 1,000,000 per c.mm. The platelets fell to 46,000 per c.mm. Six months after admission the patient developed increased intra-ocular tension with slight exophthalmos, and became almost blind. As an operation was considered inadvisable, he was treated with eserine and pilocarpine. In the last four months of his time in hospital there was some symptomatic improvement, transfusions were needed less frequently, and his blood count rose slightly. Eight months after admission his blood count was, red cells 1,700,000 per c.mm., haemoglobin 45 per cent., colour index 1.3, white cells 1,850 per c.mm. He was treated with citron, liver extract, vitamin C, vitamin A, acidophilus milk, betaxan, and 16 transfusions. He was discharged after 10 months. In the next four months he returned for three further transfusions. After this his red cell count remained stationary and his white cell count increased slowly without more transfusions. Nine months after discharge his blood count was, red cells 1,700,000 per c.mm., haemoglobin 42 per cent., colour index 1.2, white cells 5,000 per c.mm.; differential count, polymorphonuclears 22 per cent., lymphocytes 73 per cent., eosinophils 2 per cent., neutrophil myelocytes 2 per cent., normoblasts 1 per cent., and 19 months after discharge it was red cells 3,200,000, haemoglobin 70 per cent., and white cells 6,200 per c.mm.

Refractory anaemia with hypocellular marrow, followed by remission.

*Case 18.* A married Italian woman, who had been in America 10 years, first complained of pallor at the age of 24 years, eight months before the time of her admission to the Rockefeller Institute Hospital. Her family history appeared unimportant, and her diet had been a good one. For many years she had had recurrent attacks of acute bronchitis. From the age of 18 years onwards she had had mental symptoms which had been diagnosed as recurrent paranoid episodes. No history of exposure to toxic chemicals was obtained. Her menstruation had been normal and regular. Nine months before admission, after an attack of bronchitis, she complained of pallor and sore throats. Her tonsils were removed without undue haemorrhage. Six months before admission she had had some menorrhagia, and four months before admission she was found to be anaemic. Menstruation had ceased and she was found to be pregnant. Iron therapy and 34 injections of liver extract produced no improvement; a therapeutic abortion was therefore performed, and she was given five blood transfusions. She then went home, but her symptoms continued and she had occasional epistaxis.

*Physical examination.* Temperature 99.8° F., pulse 108. Well developed and well nourished woman. Pale, with distinctly yellowish skin and conjunctivae. No retinal or skin haemorrhages. Tongue normal. Chest normal. Haemic murmur. Liver and spleen just felt. No enlargement of lymphatic glands. No neurological abnormality.

*Laboratory investigations.* Blood count on admission, red cells 1,000,000 per c.mm., haemoglobin 24 per cent., colour index 1.2, mean corpuscular volume 94 cub.  $\mu$ , platelets 44,000 per c.mm., white cells 2,500 per c.mm.;



back at work, being careful to avoid further exposure to benzol, but her blood count has remained practically unaltered up to the time of writing.

Refractory anaemia, possibly associated with a poor diet and the taking of drugs of the pyrimidon-barbiturate group, with remission.

*Case 20.* A single American teacher first complained of pallor and weakness at the age of 42 years, one year before the time of her admission to the Rockefeller Institute Hospital. Her family history appeared unimportant, and apart from childhood infections and an appendicectomy she was well up till the age of 34 years. She then developed constipation, feelings of nervousness and weakness, and a tendency to blush easily. From that time onwards she took luminal, medinal, and phenolphthalein, in considerable quantities, and also at times calomel, atophan, phenacetin, and tablets containing aspirin and amidopyrine. Her diet had been a poor one, but she had not lost weight. Her menstruation was regular and normal. In the six months before admission, she was given two transfusions. Slight bleeding occurred from the gums.

*Physical examination.* Temperature 99.2° F., pulse 88. Well developed and well nourished. Tongue normal. No skin or retinal haemorrhages. Haemic murmur. No enlargement of liver, spleen, or lymphatic glands. No neurological abnormality.

*Laboratory investigations.* Blood count on admission, red cells 1,200,000 per c.mm., haemoglobin 32 per cent., colour index 1.3, mean corpuscular volume 132 cub.  $\mu$ , reticulocytes 3 per cent., platelets 48,000 per c.mm., white cells 1,750 per c.mm.; differential count, polymorphonuclear leucocytes 26 per cent., lymphocytes 64 per cent., monocytes 10 per cent., one nucleated red cell seen in counting 100 white cells. Icterus index 5 and 10 units on different occasions. Plasma bilirubin 1.5 mg. per 100 c.c. Average daily urobilinogen excretion, stools 189 mg., urine trace.

*Further course.* This patient was in the hospital for five months. She was usually afebrile with occasional fever to 99.5° F. Severe anaemia persisted and her blood count on discharge was, red cells 1,300,000 per c.mm., haemoglobin 31 per cent., white cells 1,650 per c.mm. All medication with pyrimidon or barbiturates was discontinued. She was treated with liver extract, nicotinic acid, and indol-acetic acid at different times, without evident benefit. She was also given two transfusions. After discharge the patient was treated with vitamin B<sub>1</sub> by injection thrice weekly. Her general condition and blood count slowly improved. Eleven months after discharge and one year after the last transfusion her blood count was, red cells 3,600,000 per c.mm., haemoglobin 79 per cent., colour index 1.1, white cells 5,650 per c.mm.; differential count, polymorphonuclear leucocytes 50 per cent., eosinophils 4 per cent., lymphocytes 38 per cent., monocytes 2 per cent. Up till the time of writing, six months later, she has remained clinically well and active, but her blood count has remained at almost exactly the same level.

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*Case 21.* An American chemist, 23 years old first complained of pallor and poor appetite three weeks before the time of his admission to the Rockefeller Institute Hospital. His family and previous history appeared unimportant, and his diet had been good. Up till 18 months before admission he had worked in a filling station. Since that time he had worked

in a laboratory using benzoic acid and benzol as a solvent for about two weeks each month. He last used benzol six weeks before admission. Three weeks before admission he consulted a doctor and was found to have fever and anaemia with a haemoglobin of 50 per cent. He also complained of swollen painful gums. In view of the finding of an enlarged spleen and the presence of immature white cells in blood films a diagnosis of leukaemia was made.

*Physical examination.* Temperature 100.4° F., pulse 120. Well developed and well nourished. Pale and sallow, but not jaundiced. Few old ecchymoses on thighs, no skin or retinal haemorrhages. Tongue normal, gums oedematous and swollen. Chest and heart normal. No enlargement of liver or lymphatic glands. Spleen just palpable. No neurological abnormality.

*Laboratory investigations.* Blood count on admission, red cells 2,200,000 per c.mm., haemoglobin 51 per cent., colour index 1.16, mean corpuscular volume 101 cub.  $\mu$ , reticulocytes 12 per cent., platelets 120,000 per c.mm., white cells 2,400 per c.mm.; differential count, polymorphonuclear leucocytes 16 per cent., lymphocytes 70 per cent., monocytes 14 per cent. Films, polymorphonuclears appear abnormal with poor granulation in the cytoplasm. Myelocytes (2 or 3 per cent.) were seen in some films. Icterus index 15. Average daily urobilinogen output, urine 1.5 mg., stools 570 mg. Haemolysis of red cells in saline begins at 0.42 per cent., and is complete at 0.34 per cent. (control 0.44 to 0.34 per cent.).

*Biopsy of sternal marrow.* Sections somewhat fragmented. There are sheets of densely cellular marrow with almost no fat. In some of them there are a few haemocyto blasts, numerous primary erythroblasts, and moderate numbers of normoblasts; in others there are few haemocyto blasts and numerous myelocytes with very few polymorphonuclear leucocytes. Few megakaryocytes. The marrow is a hypercellular partly mature one with erythropoiesis more active than leucopoiesis.

*Further course.* During his two weeks in hospital the patient had moderate irregular fever and his condition remained unchanged. After discharge he was treated with ascorbic acid, 125 mg. daily, and Valentine's liver extract, 90 c.c. daily by mouth. Two months after discharge his blood count was, red cells 4,000,000 per c.mm., haemoglobin 90 per cent., white cells 7,000 per c.mm., and seven months after discharge it was, red cells 5,600,000 per c.mm., haemoglobin 112 per cent., and white cells 5,900 per c.mm. The patient has avoided further exposure to benzol and has remained well; his blood count, followed for a year after discharge, remained normal.

Refractory anaemia with hypercellular partly mature marrow. Prolonged course with three remissions, one apparently after an attack of obstructive jaundice.

*Case 22.* An American Jewish schoolboy first complained of spontaneous epistaxis at the age of 17 years, 18 months before the time of his admission to the Rockefeller Institute Hospital. The family history and previous history appeared unimportant, and the patient had eaten a good diet. Epistaxis recurred at intervals, and after a few months the patient was noticeably pale. His haemoglobin was found to be 23 per cent. Three months later he was admitted to Mount Sinai Hospital. He was found to have signs of heart disease, and his spleen was palpable 4.0 cm. below the costal margin. There were a few retinal haemorrhages. His blood count was, red cells 1,100,000 per c.mm., haemoglobin 21 per cent., white cells 6,600 per c.mm.; differential count, normal; no reticulocytes seen. A biopsy of

the sternal marrow and of a lymphatic gland was performed, but no final diagnosis could be made. He was given a variety of treatments, including large amounts of liver extract, iron, and stomach preparations, and in all 12 transfusions from which he obtained temporary benefit only. During this time he continued to have severe anaemia and recurrent fever up to 104° F.

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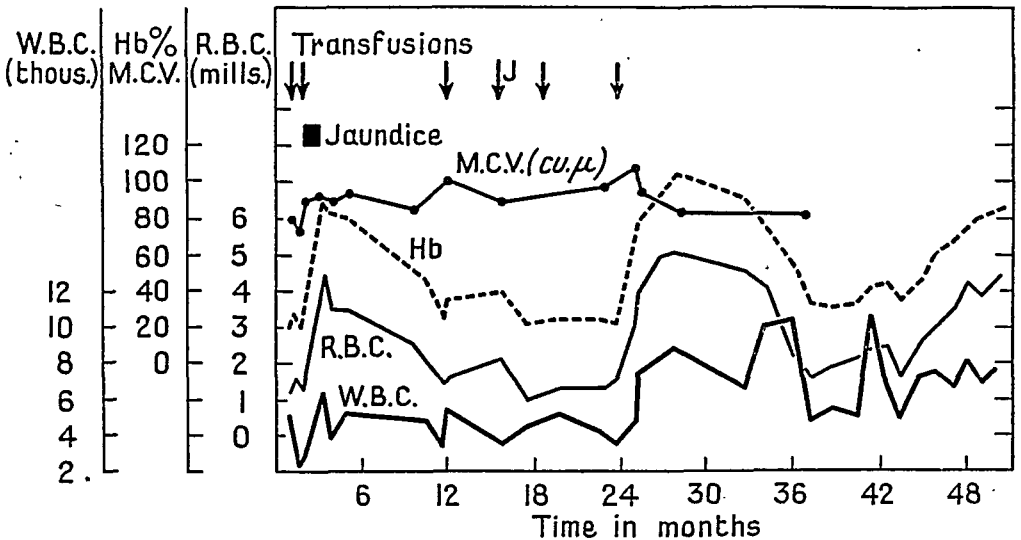


FIG. 2. Case 22. The figure shows the course of an exceptional case of refractory anaemia, in which three remissions occurred in four years. The first and most dramatic coincided with an attack of catarrhal jaundice. In the subsequent relapse a transfusion from a patient with obstructive jaundice (arrow with J in chart) was without beneficial effect. An unusual feature of this case was the absence of a significant increase in the colour index and the mean corpuscular volume during the course of the remissions.

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*Biopsy of sternal marrow.* Section from Mount Sinai Hospital made 10 months before time of admission to Rockefeller Institute Hospital. Section shows normal bone spicules separated by moderately densely hypercellular

marrow with no fat cells; slight haemosiderosis. There are haemocytoblasts, sometimes in groups, some primary erythroblasts, and conspicuous groups of basophil normoblasts, often of large size; some myelocytes, numerous polymorphonuclear leucocytes, and numerous megakaryocytes. The picture is that of a partly mature marrow with normal leucopoiesis and relatively increased erythropoiesis, the latter marked by an increase in haemocytoblasts, primary erythroblasts, and basophil normoblasts.

Rockefeller Institute Hospital section. Marrow still hypercellular without fat. Erythropoiesis conspicuous with numerous haemocytoblasts and erythroblasts, and relatively fewer normoblasts. Myelocytes, often eosinophil, in moderate numbers and few polymorphonuclears. Megakaryocytes about normal in number. The marrow is therefore similar to that in the previous biopsy, with a relative increase in immature cell forms.

*Further course.* This patient was admitted five times in the next four years (Fig. 2). During each admission he had periodic bouts of fever. At times these took the form of sudden rises to 102° or 103° F. for periods of 24 to 72 hours, sometimes with a rigor; at another time there was a typically Pel-Ebstein type of fever for a period of several months. One month after his first admission the patient had an attack of obstructive jaundice, apparently catarrhal in nature, lasting about four weeks. His blood count, which had remained almost constant at very low levels for the previous 15 months, immediately began to rise and, as shown in Fig. 2, his haemoglobin increased from 32 to 90 per cent. in 5½ weeks. At the same time his general condition improved and the spleen and lymphatic glands decreased in size, though the spleen remained palpable. The patient's subsequent course can be followed in the Figure. His blood count decreased slowly over the next seven months. A period of intramuscular liver extract therapy produced no improvement. As the remission in his disease appeared to have been associated with his attack of jaundice, he was treated in turn with 45 c.c. daily by mouth of a solution of 1 per cent. cholesterol in olive oil, with bile from 25 to 100 c.c. daily by stomach tube, and with beef bile pigment 3 gm. daily by mouth, without any significant change in his blood count. As his blood count fell, his spleen increased to its previous size. Twenty-one months after his first admission the patient was readmitted for further investigation. On physical examination at this time the cardiac signs were unchanged, the lymphatic glands in the neck, axillae, and groins were slightly enlarged, the liver was easily palpable and the spleen was enlarged almost to the umbilicus. The blood count was, red cells 1,900,000 per c.mm., haemoglobin 28 per cent., reticulocytes 1 per cent., platelets 300,000 per c.mm., white cells 3,300 per c.mm.; differential count, polymorphonuclear leucocytes 53 per cent., eosinophils 2 per cent., lymphocytes 38 per cent., monocytes 7 per cent.; films showed anisocytosis and a little poikilocytosis. The plasma bilirubin was 0.3 mg. per 100 c.c., and the average daily urobilinogen output was, stools 175 mg., urine 17.0 mg. and 28 mg. in two separate three-day periods. The increased excretion of urobilinogen in the urine suggested impaired hepatic function; X-ray examination with an opaque meal gave no evidence of oesophageal varices.

Biopsy of an axillary lymphatic gland and a further bone marrow biopsy were performed. Sections from the lymphatic gland showed extramedullary haemopoiesis, but no evidence of Hodgkin's disease. The specimen of marrow removed at this time was densely hypercellular, with numerous haemocytoblasts often in groups and relatively fewer erythroblasts and normoblasts; numerous myelocytes, often eosinophil, and very few polymorphonuclear

leucocytes. No megakaryocytes seen. The marrow was thus similar in type to that in previous biopsies, with a relative predominance of erythropoiesis, but it had become progressively more immature. At this time the patient had a swinging fever with peaks recurring weekly and intervening periods of subnormal temperature. This began about four months before his second admission and was still present when he was discharged three months later. He was discharged unimproved on ferrous sulphate 0.6 gm. twice daily. Almost immediately he had a second remission as shown in Fig. 2, and his haemoglobin increased in four months from 22 to 104 per cent. It seems unlikely that the iron medication was responsible for this increase. At the same time his fever practically disappeared, and his spleen receded to within three fingers breadth of the costal margin. Slight enlargement of the lymphatic glands persisted. His blood count decreased slowly over the next seven months, and then more rapidly, and was at low levels for a further six months. Forty-four months after his first admission, and about five years after his first symptoms a third remission began, apparently quite spontaneously, and has continued up till the time of his last visit.

### *Summary*

A series of 58 cases of anaemia, which proved resistant to any form of treatment except blood transfusions, was divided according to the histological appearances in sections of the bone marrow into four groups.

1. Refractory anaemia with partly mature cellular marrow, or Pseudo-aplastic anaemia (31 cases). In this group the bone marrow differed least from the normal, symptoms were relatively mild, the illness was sometimes of long duration, and spontaneous remissions were not uncommon. Leucopenia and thrombocytopenia were inconspicuous or absent, and haemorrhages and infections occurred infrequently. Pigmentation of the skin was seen as a complication, and less frequently haemochromatosis. In some cases this type of anaemia appeared to be a temporary phase in the development of other disorders of the haemopoietic system.

2. Refractory anaemia with hypoplastic marrow, or Aplastic anaemia (11 cases). In this group, as compared with the previous one, the duration of the illness was usually shorter, symptoms, especially haemorrhage, were more severe and spontaneous remissions occurred infrequently. Leucopenia and thrombocytopenia were present in some degree in all cases.

3. Refractory anaemia with immature cellular marrow, or Chronic granulocytopenia (12 cases). In this group the bone marrow contained very large numbers of erythroblasts, and on superficial examination presented an appearance not unlike that of lymphatic leukaemia. There was usually no more than moderate anaemia, but severe and progressive granulocytopenia, often with considerable numbers of immature white cells in the peripheral blood stream. The average duration of the illness was relatively short, and the most prominent clinical feature was the occurrence of areas of necrosis and infection, particularly in the neighbourhood of the mouth and anus. A remission occurred in one case only.

4. Refractory anaemia with fibrosis, sclerosis and giant cell hyperplasia of the marrow, or Myelosclerosis (4 cases). In this group the duration of the

illness was relatively short and the outcome uniformly fatal. The most prominent clinical feature was a progressive and considerable enlargement of the spleen and liver.

The onset of a remission in refractory anaemia is usually indicated by an increase in the white cell count, the colour index, and the mean corpuscular volume. Remissions may be complete or incomplete. When they are incomplete the blood picture comes to resemble closely that seen in the mild macrocytic anaemia of liver disease.

Acknowledgements and references will be found at the end of Part II.

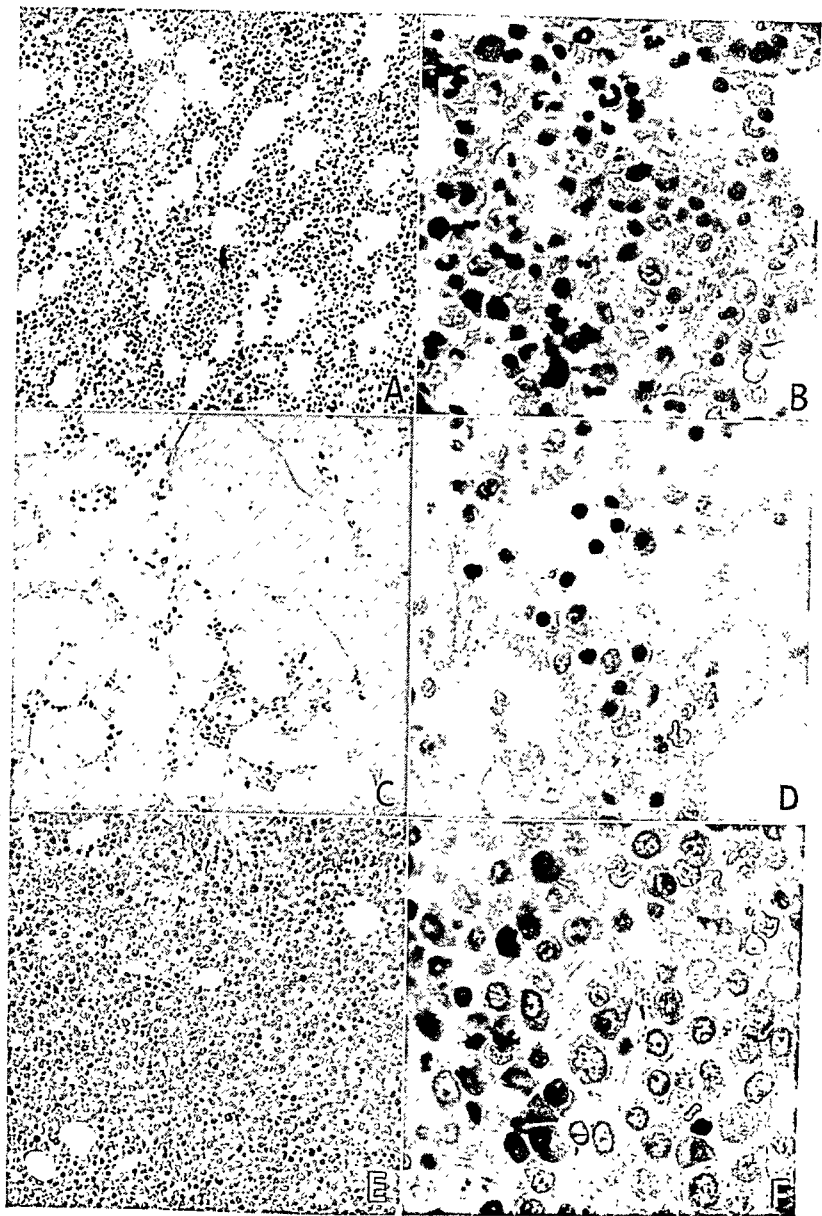


FIG. 3. Sternal marrow of normal cellularity. Magnification A  $\times 125$ , B  $\times 500$ . Sternal marrow in refractory anaemia with hypocellular marrow. Magnification C  $\times 125$ , D  $\times 500$ . Sternal marrow in refractory anaemia with immature cellular marrow. Magnification E  $\times 125$ , F  $\times 500$ .

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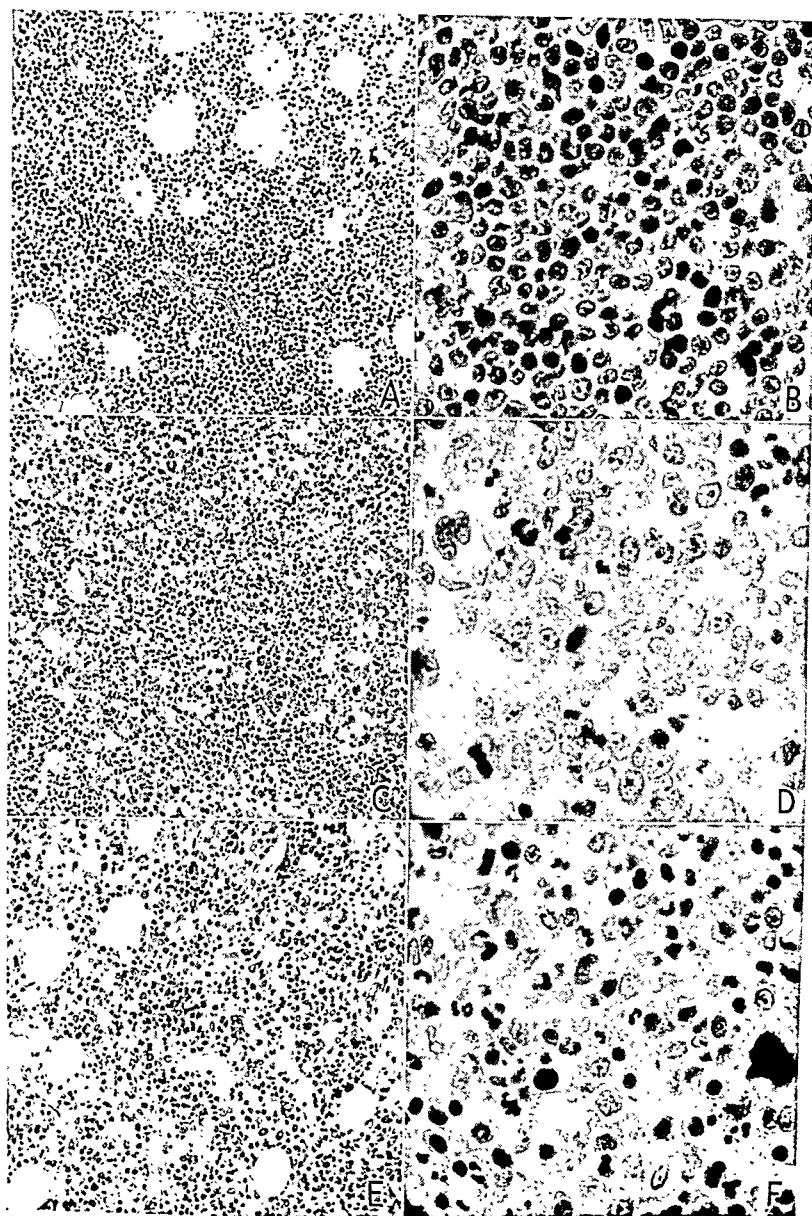


FIG. 4. Sternal marrow in lymphatic leukaemia (for comparison with Plate 13, Fig. 3, E and F). Magnification A  $\times 125$ , B  $\times 500$ .  
Sternal marrow in myeloid leukaemia (for comparison with Fig. 4, E and F). Magnification C  $\times 125$ , D  $\times 500$ .  
Sternal marrow in refractory anaemia with partly mature cellular marrow. Magnification E  $\times 125$ , F  $\times 500$ .

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age incidence of the whole group, and we are unable to confirm the statement frequently made that aplastic anaemia is a disorder of children and young adults.

*Anaemia in near relatives.* In one instance only was there a history of anaemia before the onset of the present symptoms. The patient was a child who was said to have been anaemic from haemorrhage at birth. In two cases sisters of a patient had died of anaemia of unknown nature at the ages of 21 and 40 years. In another case a brother had died 18 years previously of an illness attributed to exposure to benzol, at a time when both patient and brother were exposed to benzol for a period. In two cases the mother of the patient was said to be 'anaemic', but this seemed doubtful. There is therefore no evidence for a hereditary predisposition to anaemia in the families of these patients.

*Possible associated conditions. Endocrine disturbances.* Four patients had a history of an endocrine disorder; two were eunuchs, and two had suffered from hyperthyroidism. In five cases there was a history of an endocrine disorder in near relatives. One patient developed haemachromatosis with diabetes during the course of his anaemia, but this appeared to be a complication of the anaemia. In four women the first symptoms of their disorder appeared at or very near the menopause; in two more there was a history that the symptoms were more severe at or shortly before the time of menstruation. There is no positive evidence of any relationship to endocrine disorder in these figures, but the association with eunuchoidism, the menopause, and menstruation merits further investigation.

### *Case Reports*

Refractory anaemia in a eunuch; apparently hypercellular predominantly erythropoietic marrow.

*Case 23.* An American-born waiter of Polish descent first complained of weakness and pallor at the age of 21 years, five years before admission to the Rockefeller Institute Hospital. His father had died of tuberculosis, but his family history otherwise seemed unimportant. At the age of 15 years the patient had had an operation for the repair of bilateral inguinal herniae; the left testicle was found to be incarcerated, engorged, and haemorrhagic, and was removed. The right testicle was undescended and was not seen at the operation. Sexual development ceased from this time. Ten years before admission the patient had worked for two years in a printing-shop where benzol was used to clean the type. Again, for three months almost five years before admission he was exposed to benzol fumes as a waiter in a restaurant. It seems likely, however, that the first symptoms of his anaemia were present before this time. Shortly after his first definite complaint he was admitted to the New Britain Hospital, and his blood count was found to be, red cells 3,100,000 per c.mm., haemoglobin 38 per cent., white cells 8,000 per c.mm. There was no enlargement of the spleen or lymphatic glands. He was treated with liver extract, and his condition improved considerably. Three years later he was again in the New Britain Hospital with severe anaemia, occasional epistaxis, and difficulty in vision from retinal haemorrhages. He was in hospital 11 months, and received 46 transfusions.

During this time he developed a finely granular brown pigmentation of the skin.

*Physical examination.* Temperature 98.2° F., pulse 116. Well developed and well nourished. No facial or chest hair, and pubic hair under-developed. Brown pigmentation of skin, especially on exposed surfaces. No haemorrhages. No jaundice. Tongue normal. No enlargement of lymphatic glands. Haemic murmur. Blood-pressure 130/50. Liver and spleen just palpable. Bilateral herniotomy scars. No testicles palpable. No neurological abnormality.

*Laboratory investigations.* Blood count on admission, red cells 1,200,000 per c.mm., haemoglobin 23 per cent., colour index 1.0, reticulocytes 0.8 per cent., platelets 252,000 per c.mm., white cells 1,300 per c.mm.; differential count, polymorphonuclear leucocytes 56 per cent., lymphocytes 44 per cent. Icterus index 3. Haemolysis of red cells in saline began at 0.42 per cent., and was complete at 0.26 per cent. (control 0.44 to 0.34 per cent.). Test meal, free hydrochloric acid present. Twenty-four hourly urobilinogen output, urine 1.3 mg., stools 272 mg.

*Biopsy of sternal marrow.* Apparently hypercellular, but comparison is difficult as normal architecture is quite lost. Large areas of pink structureless material and of haemorrhage interspersed with densely cellular areas without fat, some predominantly erythropoietic with conspicuous groups of haemocyto blasts, numerous erythroblasts, and few normoblasts, others contain a few myelocytes and polymorphonuclear leucocytes. Few megakaryocytes. Erythropoiesis therefore predominates over leucopoiesis. Considerable amounts of conspicuous golden-brown pigment inside and outside phagocytes.

*Further course.* During the patient's stay in hospital he had slight irregular fever. After transfusions his blood count returned to its previous level in a few days. Treatment with androsterone and testosterone in large doses had no effect. He died six weeks after admission. No autopsy was allowed.

Refractory anaemia with severely hypocellular marrow in a eunuch, with remission.

*Case 24.* An American stone-cutter, aged 43 years, first noticed dyspnoea on exertion nine months before the time of his admission to the Rockefeller Hospital. The patient had lived all his life in Virginia, and his family history appeared unimportant. His diet had been good, and there was no history of any exposure to toxic substances. He was a eunuch, apparently from birth; apart from this he had had no illnesses other than childhood fevers. A month after his first symptom the extraction of two teeth was followed by severe haemorrhage. He became pale, developed petechial haemorrhages on the arms and legs, and was found to be severely anaemic. He was treated with liver, iron, and transfusions with no more than temporary benefit.

*Physical examination.* Temperature 99° F., pulse 106. Tall and poorly nourished, with slender limbs of disproportionate length and wide hips. Dry scaly skin, with brown pigmentation of legs. Fine scanty hair on face; pubic hair of feminine distribution. Numerous petechial haemorrhages on abdomen, arms, and legs; no retinal haemorrhages. Haemic murmur. Blood-pressure 122/65. No enlargement of liver, spleen, or lymphatic glands. Penis infantile, no testicles palpable. No neurological abnormality.

*Laboratory investigations.* Blood count on admission, red cells 1,700,000 per c.mm., haemoglobin 40 per cent., colour index 1.2, mean corpuscular volume 100 cub.  $\mu$ , reticulocytes 3.9 per cent., platelets 62,000 per c.mm.,

white cells 2,950 per c.mm.; differential count, polymorphonuclear leucocytes 38 per cent., lymphocytes 56 per cent., monocytes 2 per cent., eosinophils 3 per cent., myelocytes 1 per cent. Fragility of red cells in saline normal. Test meal, free hydrochloric acid present after alcohol and histamine. Barium meal, no abnormality. Radiograph of skull, sella normal. Occult blood test on faeces, negative.

*Biopsy of sternal marrow.* Section shows severely hypocellular marrow with scattered groups of primary erythroblasts and normoblasts; occasional myelocytes and polymorphonuclears. Small amounts of golden-yellow pigment.

*Further course.* No details are available. A year later he was said to be 'doing better', and six years later he was known to be clinically well and active.

Refractory anaemia with onset at the menopause, in a patient exposed to benzol; hypoplastic marrow; laboratory evidence and some post-mortem evidence of haemolysis.

*Case 25.* A German-born housewife, who had been 20 years in America, first complained of nose-bleeding and bruising easily 11 months before the time of her admission to the Rockefeller Institute Hospital. At the age of 31 years she had had one ovary and both ovarian tubes removed, apparently for inflammation. Her last period had occurred four months before the time of her first symptom. Her father had mild diabetes, but the remainder of the family history seemed unimportant. The patient's diet was unusual; for five years she had eaten no red meat, and for 15 years no fresh fruit or vegetables; she had, however, eaten cooked fruits and vegetables. For many years the patient had occasionally used benzol for cleaning purposes, and for the last eight years she had done all her own cleaning, buying the benzol by the gallon and doing the work in a small bathroom about once every two weeks. At times this would make her feel ill, and she would have to leave the bathroom. She used benzol for the last time four months before the date of admission; on this occasion she felt very sick and almost fainted. She concluded that the benzol might be harming her, and gave up using it. She became gradually weaker, and her dyspnoea on exertion increased. Crops of petechiae and bleeding from the gums appeared. She also complained of aching in the bones. She was treated with liver extract, arsenic, and iron.

*Physical examination.* Temperature 99.4° F., pulse 96. Slightly pale and moderately obese. Numerous fine petechiae and a few ecchymoses on skin; petechiae on buccal mucous membrane. No retinal haemorrhages. Gums moderately retracted, tongue normal. Haemic murmur. Blood-pressure 150/80. No enlargement of liver, spleen, or lymphatic glands. No neurological abnormality.

*Laboratory investigations.* Blood count on admission, red cells 2,500,000 per c.mm., haemoglobin 60 per cent., colour index 1.2, mean corpuscular volume 105 cub.  $\mu$ , reticulocytes varied between 2.0 and 7.0 per cent., platelets 30,000 per c.mm., white cells 3,850 per c.mm.; differential count, polymorphonuclear leucocytes 62 per cent., lymphocytes 29 per cent., monocytes 8 per cent., eosinophils 1 per cent. Films, anisocytosis, little poikilocytosis, occasional macrocytes. Red cell fragility, haemolysis in saline began at 0.46 per cent. and was complete at 0.36 per cent. (control 0.44 to 0.34 per cent.). Icterus index varied between 5 and 20. Plasma bilirubin 0.4 mg. per 100 c.c. Twenty-four hourly urobilinogen output, urine trace, stools 232 mg.

Wassermann reaction negative. Blood ascorbic acid 1.75 mg. per 100 c.c. Barium meal and radiographs of chest and long bones normal.

*Sternal bone-marrow biopsy.* Marrow severely hypocellular. There are islands of very active erythropoiesis with haemocytoblasts, primary erythroblasts and normoblasts. In other areas there are normoblasts, myelocytes, and polymorphonuclears. Erythropoiesis much more active than leucopoiesis.

*Further course.* The patient remained in the Rockefeller Hospital for three months. She had slight irregular fever, and continued to have petechiae and slight bleeding. Treatment with transfusions, liver, and stomach preparations by mouth, and large amounts of acidophilus milk produced no improvement. Her blood count on discharge was, red cells 1,800,000 per c.mm., haemoglobin 44 per cent., platelets 50,000 per c.mm., white cells 3,750 per c.mm.; differential count, polymorphonuclear leucocytes 23 per cent., lymphocytes 72 per cent., monocytes 2 per cent., eosinophils 3 per cent. One month later the patient was admitted to the New York Hospital. She continued to bleed from the nose and gums, and into the skin, conjunctivae, and fundi. Seven transfusions in 19 days increased her haemoglobin to 83 per cent., but a week later it had decreased again to 35 per cent. Five weeks after admission the patient either fell or jumped from the window of her room and was killed instantaneously.

*Summary of autopsy report* (from Department of Pathology, New York Hospital). Middle-aged obese woman. Multiple fractures, recent internal and external lacerations and skin abrasions. Numerous petechiae and ecchymoses. Pleural, pericardial, and peritoneal petechiae. Atheroma of aorta. Spleen (150 gm.) with reddish-purple cut surface and prominent markings. Liver (1,800 gm.) soft with yellowish-brown cut surface. Haemorrhage into pelves of both kidneys. Sub-mucosal petechiae in bladder. No enlarged lymphatic glands. Vertebral and sternal marrow yellowish-pink with irregular darker red areas. Femoral marrow pinkish-yellow with large bright yellow areas.

*Summary of microscopical findings* (from Department of Pathology, New York Hospital). Vertebral, femoral, and sternal marrow extremely hypocellular. There are numbers of small round cells, in shape and size resembling lymphocytes. There is a great decrease in leucogenesis and erythrogenesis, and there are no megakaryocytes in the sections. There are moderate numbers of large mononuclear cells containing a golden-brown pigment. In the liver cells there are small and large clear vacuoles containing a substance which stains red in a frozen section stained with Sudan III. Spleen, distinct malpighian bodies, and pulp containing few erythrocytes. Many large mononuclear cells contain engulfed erythrocytes. Heart muscle, deposits of golden-brown pigment about the nuclei in many of the muscle-bundles.

Refractory anaemia with recurrent episodes of haemolysis, sometimes accompanying menstruation. Hypercellular immature predominantly erythropoietic marrow.

*Case 26.* An American girl, aged 15 years, first became unwell after a brief attack of nausea and vomiting five months before her admission to the Rockefeller Hospital. Her family and previous history appeared unimportant. Her menses had begun at the age of 12 years and had appeared regularly every 28 days, until the onset of her illness, since when she had had amenorrhoea. Shortly after her first complaint she was treated with injections of liver extract, but became paler. She was admitted to a hospital and given

five transfusions without permanent benefit. She had noticed that exposure to bright sunlight caused her to feel ill with nausea, fever, and headache.

*Physical examination.* Temperature 98·4°F., pulse 112. Well developed and well nourished, but pale and sallow. Tongue normal. No skin or retinal haemorrhages. Slight enlargement of cervical lymphatic glands, but no enlargement of other lymphatic glands. Soft haemic murmur. Liver palpable three fingers breadths below costal margin; spleen easily palpable under costal margin. No neurological abnormality.

*Special investigations.* Blood count on admission, red cells 1,100,000 per c.mm., haemoglobin 19 per cent., colour index 0·86, mean corpuscular volume 85 cub.  $\mu$ , reticulocytes 0·6 per cent., platelets 244,000 per c.mm., white cells 1,900 per c.mm.; differential count, polymorphonuclear leucocytes 50 per cent., lymphocytes 48 per cent., monocytes 2 per cent. Anisocytosis and poikilocytosis. Icterus index 6. Fragility of red cells, haemolysis began in 0·44 per cent. and was complete in 0·34 per cent. saline. Blood-urea nitrogen 10·5 mg. per 100 c.c. Blood amino-nitrogen 5·59 mg. per 100 c.c. Average daily urobilinogen excretion in stools, 213 mg.

*Sternal marrow biopsy.* Normal bone spicules and areas of hypercellular marrow with normal architecture alternating with pink structureless areas. Cellular areas consist almost entirely of immature basophil cells and primary erythroblasts, with occasional groups of normoblasts. Very few myelocytes, no polymorphonuclears, and few megakaryocytes seen.

*Further course.* This patient was in the hospital on five occasions during the next 12 months. During four of them she had irregular periods of fever, usually rising to a peak of 103°F. and separated by intervals of normal or subnormal temperature, an irregular Pel-Ebstein type of fever. With each bout of fever the patient felt ill, with a headache and a coated tongue. At the same time there was an increase in the size of the spleen, an increase in the plasma bilirubin, and an increase in the amount of urobilinogen excreted in the urine and faeces. It therefore seemed evident that the patient was experiencing recurrent episodes of haemolysis. At the same time there was a pronounced increase in the amount of non-glucose reducing substance excreted in the urine, a finding which would be consistent with the appearance in the circulation at these times of a toxic substance, requiring conjugation as a glucuronate. It was noticed that on the occasions when the patient menstruated, one of the episodes usually occurred immediately before the period, with improvement when the period was over; at other times, she had a haemolytic episode, but no uterine bleeding, at the time when her period was due. In view of this observation, it was thought wise to attempt a temporary sterilization with X-rays. The patient was given one of four projected X-ray treatments to the pelvis. Six days later she was found to have a profound leucopenia with only 650 white cells per c.mm. and slight jaundice with an icterus index of 20. Further irradiation was therefore abandoned. In between these episodes the spleen decreased in size and at times could not be felt, but at other times the patient's condition changed little. Her red cell count was usually between 1,000,000 and 2,000,000 per c.mm., haemoglobin between 20 and 30 per cent., and white cells from 1,000 to 3,000 per c.mm., and the platelet count varied from 100,000 to 300,000 per c.mm. A few myelocytes were frequently present in the films and occasionally a number of normoblasts were seen. A sternal puncture one year after her first admission was interpreted as showing a hypocellular marrow with the following differential count, polymorphonuclear leucocytes 27 per cent., myelocytes 16 per cent., myeloblasts 1 per cent., eosinophils 2 per cent.,

lymphocytes 7 per cent., normoblasts 27 per cent., erythroblasts 4 per cent., megaloblasts 4 per cent., primitive cells 1 per cent. At the time of writing, 21 months after her first admission, the patient's condition remains almost unchanged.

*Allergy.* There was a history of allergic disorders in three patients and in near relatives of three other patients.

*Gastro-intestinal disorders.* There was a history of gastro-intestinal disorder antedating the symptoms of anaemia in six cases. This appeared usually to have been a functional dyspepsia. Gastric analyses were performed in 37 cases. There was complete achlorhydria in two cases only; in the remaining 35 free hydrochloric acid was present in the resting specimen of stomach contents in 15 cases, in the specimen obtained after the administration of alcohol in 12 cases, and in the specimen obtained after the administration of histamine in 8 cases. The incidence of achlorhydria (5.4 per cent.) in these cases is well below the normal figure after histamine of 11 per cent. given by Ruñin and Dick (1939).

*Disorders of the tongue, nails, and nervous system.* Some atrophy of the papillae of the tongue was present in seven cases, being slight and limited to the edges; severe atrophy, such as that seen in pernicious anaemia, was not seen in any of these cases. Dysphagia was present in one case. Koilonychia was never seen. There was no definite sign of organic nervous disease in any case.

*Other conditions.* In one case there was a history of haemorrhagic purpura, for which a splenectomy had been performed, antedating by many years the symptoms of anaemia; in three other cases there was a history of unusually easy bruising for many years before the onset of symptoms. In nine patients there was a history of chronic ear, nose, or sinus infections at some time before the onset of symptoms. Such conditions are very common in New York hospital practice, and it seems unlikely that these had any aetiological significance. In five cases there was a history of rheumatic disorders including 'arthritis' and acute rheumatism.

*Exposure to potentially toxic substances.* It is generally accepted that refractory anaemia may be caused by exposure to benzol, arsenobenzol, X-irradiation, and radioactive substances. The potentially toxic substances to which our patients had been exposed are shown in the Table. In a few cases the clinical history left little doubt that the substance in question was concerned in the production of the illness; in the majority there was nothing to prove conclusively such a connexion.

The difficulties involved in assessing the effect of toxic substances on the blood-forming organs have been discussed by Witts (1936), and Fitz-Hugh (1938). As Witts points out, much can be learned from a study of pyrimidon agranulocytosis and other proven varieties of poisoning affecting the haemopoietic system. Firstly, if a number of persons is exposed to a constant and poisonous dose of a toxic substance, the majority will usually develop symptoms of the form of poisoning ordinarily associated with the substance, but with varying degrees of severity. Thus the persons exposed exhibit a varying

susceptibility. Secondly, an occasional person exposed to the same toxic substance will develop quite a different train of symptoms, that is, will exhibit an idiosyncrasy to the substance. This idiosyncrasy may follow the taking of a small dose which would be quite harmless to the majority of persons. Thirdly, there is evidence in the case of amidopyrine and one or two other drugs, that a person may become sensitized, so that after a certain amount has been taken without untoward event, a subsequent dose may be followed by alarming symptoms. Lastly, symptoms may not immediately follow the taking of a toxic substance, but may be considerably delayed.

In the case of anaemia produced by toxic substances, it is hardly possible to prove that a given substance was responsible for the anaemia, since it would be unjustifiable to submit the few cases that recover to a test dose of the suspected substance, and no other satisfactory test of susceptibility has been devised. It is not unlikely, however, that the principles mentioned above as applying to the effects of amidopyrine on the leucopoietic system, also apply to the effects of toxic substances on erythropoiesis. If this is the case, certain grounds for denying the significance of exposure to a toxic substance are invalid. Exposure to a toxic substance cannot be excluded as a responsible factor on the grounds that large numbers of people were exposed to the same substance and dose, without developing anaemia, nor on the grounds that anaemia is not the usual symptom produced by an overdose of the substance, nor on the grounds that the anaemia came on at an interval after exposure to that substance had ceased. In most instances it is impossible in an individual case either to prove or disprove the importance in aetiology of exposure to a toxic substance.

Whatever be the merits of scepticism from an academic point of view, the position from a practical point of view is clear. It is of paramount importance that patients with refractory anaemia should be removed from exposure to any possibly toxic substance, however remote the possibility. At the moment no other single measure has so great an effect on prognosis. Patients should therefore be questioned closely about their food, their use of drugs, toilet or cosmetic preparations, and their home and work environment. Exposure to any potentially toxic substance, in particular to volatile organic vapours and to drugs containing the benzene ring, should be regarded with suspicion, and terminated. The following case, the least severe in the series, is included since we believe that it is an example of the earliest effects of exposure to a toxic substance, and illustrates the good prognosis if such exposure is terminated early.

Mild macrocytic anaemia, possibly associated with the use of paraphenylenediamine hair dye.

*Case 27.* An unmarried opera singer first complained of undue fatigue three years before the time of her admission to the Rockefeller Institute Hospital at the age of 46 years. Her family history and previous history appeared unimportant, except that the menopause had occurred at the time of the onset of her symptoms. Since then she had been gaining weight. For three years she had used a hair dye known to contain paraphenylenediamine.



diamine, and had developed an erythematous eruption at the hair margin, which became worse when she used the dye. She had had no bleeding and no other symptoms.

*Physical examination.* Temperature 98·0° F., pulse 62. Erythematous eruption at the hair margin behind the left ear. No other abnormality discovered.

*Laboratory investigations.* Blood count on admission, red cells 3,800,000 per c.mm., haemoglobin 90 per cent., colour index 1·2, mean corpuscular volume 104 cub.  $\mu$ ., reticulocytes 1·3 per cent., white cells 6,200 per c.mm.; differential count, polymorphonuclear leucocytes 72 per cent., lymphocytes 24 per cent., monocytes 2 per cent., eosinophils 2 per cent. Films showed macrocytosis. Icterus index 7 and 10 on different occasions. Plasma bilirubin 0·8 mg. per 100 c.c. Test meal, free hydrochloric acid present in all specimens. Barium meal and cholecystogram normal.

*Further course.* The patient was advised to give up the use of the hair dye, and was treated with liver extract. After three months her blood count was, red cells 4,400,000 per c.mm., haemoglobin 89 per cent., colour index 1·0, mean corpuscular volume 91 cub.  $\mu$ ., white cells 8,500 per c.mm. No further blood counts are available, but the patient is alive and well two years after the time of her admission to hospital.

Of the 66 patients in this series, 24 were known to have been exposed to a potentially toxic substance (see Table). Of the patients exposed to benzol,

TABLE

*Patients Exposed to Potentially Toxic Substances*

Benzol . . . . .	11
Arsphenamine . . . . .	2
Radioactive paint . . . . .	2
X-rays and radium . . . . .	1
Benzol and arsphenamine . . . . .	1
A volatile insecticide . . . . .	2
A paraphenylene diamine hair dye . . . . .	3
Hydroquinone . . . . .	1
Resin . . . . .	1
Atophan . . . . .	2
Known analgesic drugs, including allonal, pyramidon, acetanilid, phenacetin, and a theobromine-phenobarbital compound . . . . .	4
Unknown sedatives and analgesics . . . . .	3
Creosote . . . . .	1
	<hr/> 34

four were working together in a rotogravure printing shop, where an ink solvent containing benzol was employed. The air in the shop was found to contain 24 to 1060 parts of benzol per million parts of air, and the men also cleaned their hands with benzol. Another patient was a hairdresser, who began three months before the onset of her symptoms to use a 'rapid hair cleaner' containing 30 per cent. of benzol for shampoos. When she used the preparation on her own hair she felt nauseated and ill. Another had worked 10 years before the onset of his symptoms in a printing shop where benzol was used to clean the type; shortly before the onset of his symptoms he was again exposed to benzol fumes when working in a restaurant. This was possibly an example of sensitization. Another, because of straitened circumstances, began to do her own cleaning and used 'gallons' of benzol in a small

bathroom; the fumes made her feel sick, so that she had to leave the room and lie down to recover before she could go on with her work. Another worked in an artificial leather plant using a solvent containing benzol. Another had been exposed in a rubber manufacturing plant to fumes of benzol, but not for four years before the onset of symptoms.

The patients exposed to arsphenamine had received the drug as treatment for syphilis. In one case the patient had had two previous courses four and 12 years previously. One month before the onset of his symptoms he was given a single dose of arsphenamine which was followed immediately by headache, dyspnoea, and substernal pain, and he was in bed for several days. This seems to be another possible example of sensitization. In spite of these symptoms he was given eight further injections. He was then given two transfusions and subsequently a further injection of arsphenamine. This in his own words made him 'deathly sick', and he was admitted to a hospital.

The patients exposed to radioactive paint were luminous watch-dial painters, who 'pointed' their brushes with their lips; both died with anaemia and one is known to have developed osteogenic sarcomata. The patient exposed to X-rays and radium had been a therapeutic radiologist for nine years. Seven months before the onset of his symptoms he had bought a new and more powerful apparatus.

Two patients had been exposed to a volatile organic insecticide, both had used the preparation in unusual amounts, and had 'soaked' their pillows with it. In one patient (Case 33) a history of the occurrence of symptoms, including bleeding during periods when the preparation was used, and of freedom from symptoms in periods when it was not used, seems to incriminate this preparation beyond doubt. A third patient not included in this series had been exposed to excessive amounts of this insecticide, to a 'rapid cleaner' containing 30 per cent. of benzol, and to a hair dye, though the nature of the last is not known. Yet another patient not included in this series had regularly sprinkled the shelves of his wardrobe with paradichlorbenzol to kill moths.

Nine patients had taken drugs containing the benzene ring—two had taken atophan, four had taken such preparations as allonal, pyramidon, acetanilid, phenacetin, and a theobromine phenobarbital compound, and three had taken analgesic medication of unknown nature. With the exception of atophan, the drugs had been taken in large amounts for long periods. In two of these patients, both of them cases in which the nature of the drug taken was not known, a febrile reaction followed the taking of a single dose of allonal in one case and pyramidon in the other, while the patients were under observation in the hospital. In two others the beginning of a remission appeared to follow the cessation of all medication. In the remaining cases it can only be a matter of opinion whether the taking of drugs had any aetiological significance. Severe anaemia in two, and mild anaemia in 11, of 15 epileptic patients under prolonged treatment with barbiturates has been observed by Maillard and Jammet (1937). It must also be a matter of

opinion whether exposure to hydroquinone, resin dust, and creosote respectively in the remaining three patients was significant. In the case of resin dust, the possibility is strengthened by the fact that two further patients exposed to fumes of a solder impregnated with resin have been seen in this department suffering from malaise, fever, and bleeding gums with a white cell picture resembling that of infectious mononucleosis.

Apart from the three examples of immediate reaction to drugs containing the benzene ring so far mentioned in patients already anaemic (one each to allonal, pyramidon, and arspenamine), three other patients gave a history of similar occurrences. One at the time of onset of his symptoms was given a duodenal lavage with salvarsan for the treatment of *Giardia lamblia*, and this was followed by fever, a rigor, and urticaria. Another was given a number of injections of pentnucleotide, each of which was followed by a rigor. A third was given a course of pills 'for her heart', the taking of which was frequently followed by a rigor. A fourth patient, to be mentioned later, developed an increasing leucopenia and died after two doses of pyramidon given in hospital.

### Case Reports

Moderate anaemia in a printer exposed to benzol; severely hypocellular predominantly erythropoietic marrow with slight haemosiderosis.

*Case 28.* A Jewish printer, aged 33 years, was referred to the Rockefeller Institute Hospital by the Department of Labour of the State of New York. His past history and family history seemed unimportant. He had been exposed to benzol fumes at his work, and had noticed one or two ecchymoses; otherwise he felt perfectly well. His diet had been good.

*Physical examination.* Temperature 98° F., pulse 88. Well developed and with peculiar yellowish pallor. Few subcutaneous ecchymoses, and flicking of the skin with a finger caused further small ecchymoses. No retinal haemorrhages. Tongue and throat normal. Chest and heart normal. No enlargement of liver, spleen, or lymphatic glands. No neurological abnormality.

*Laboratory investigations.* Blood count on admission, red cells 2,100,000 per c.mm., haemoglobin 63 per cent., colour index 1.5, mean corpuscular volume 95 cub.  $\mu$ , reticulocytes 4.0 per cent., platelets 42,000 per c.mm., white cells 1,750 per c.mm.; differential count, polymorphonuclear leucocytes 72 per cent., lymphocytes 16 per cent., monocytes 12 per cent. Film shows anisocytosis, poikilocytosis, and macrocytosis. Icterus index 5. Haemolysis in saline began at 0.5 per cent. and was complete at 0.34 per cent. (control 0.46 to 0.28 per cent.). Test meal, free hydrochloric acid present after histamine. Wassermann reaction negative. Plasma bilirubin 1.4 mg. per 100 c.c. Twenty-four hour urobilinogen output (average of three 3-day periods), urine 4.2 mg., stools 154 mg.

*Biopsy of sternal marrow.* Severely hypocellular. Areas of fat and haemorrhage with occasional strands and groups of cells which are mostly erythrocytes and polymorphonuclear leucocytes. No megakaryocytes seen. Small deposits of haemosiderin.

*Further course.* During the patient's two weeks in hospital he was treated with nicotinic acid, which appeared to reduce his total urobilinogen output to 95 mg. per diem. After discharge he was treated with liver extract and

betaxan. Five months later his blood count was, red cells 4,300,000 per c.mm., haemoglobin 101 per cent., colour index 1.2, mean corpuscular volume 92 cub.  $\mu$ ., reticulocytes 4.4 per cent., platelets 124,000 per c.mm., white cells 3,450 per c.mm.; differential count, polymorphonuclear leucocytes 44 per cent., lymphocytes 38 per cent., monocytes 16 per cent., basophils 2 per cent.

Moderate anaemia in a printer exposed to fumes of benzol; slightly hypercellular, slightly immature, predominantly erythropoietic marrow; haemosiderosis in marrow.

*Case 29.* A printer, 26 years old, of Italian extraction, first felt unusually tired two months before admission. He had had no previous illness of importance, and his diet had been a good one. At his work he was exposed to the fumes of an ink solvent containing benzol. He complained of dizziness and palpitation, and one week before admission of epistaxis and some bruises on his arms.

*Physical examination.* Temperature 99.8 °F., pulse 124. Of small stature, but well developed and well nourished. Few ecchymoses on arms and legs, but no other haemorrhages. Tongue normal. No enlargement of liver, spleen, or lymphatic glands. No neurological abnormality.

*Laboratory investigations.* Blood count on admission, red cells 2,600,000 per c.mm., haemoglobin 62 per cent., colour index 1.2, mean corpuscular volume 94 cub.  $\mu$ , reticulocytes 5.2 per cent., platelets 28,000 per c.mm., white cells 5,650 per c.mm.; differential count, polymorphonuclear leucocytes 88 per cent., lymphocytes 8 per cent., monocytes 4 per cent. Occasional normoblasts and myelocytes in stained films. Haemolysis of red cells in saline began at 0.50 per cent., and was complete at 0.34 per cent. (control 0.46 to 0.28 per cent.). Test meal, free hydrochloric acid present. Plasma bilirubin 1.0 mg. per 100 c.c. Twenty-four hourly urobilinogen output (average of three 3-day periods), urine 1.6 mg., stools 86 mg.

*Sternal marrow biopsy.* Normal architecture with less fat than normal. Marrow evenly and fairly densely cellular with numerous erythrocytes; normoblasts, and polymorphonuclears. There is a moderate relative increase of immature cells. Few megakaryocytes. Erythropoiesis more active than leucopoiesis. Some pigment present in reticulo-endothelial cells.

*Further course.* The patient was removed from exposure to benzol, and treated at different times with nicotinic acid, liver extract, and betaxan. His symptoms disappeared, he gained weight, and six months after he was first seen his blood count was, red cells 3,300,000 per c.mm., haemoglobin 100 per cent., colour index 1.5, reticulocytes 5 per cent., platelets 152,000 per c.mm., white cells 8,300 per c.mm.; differential count, polymorphonuclear leucocytes 56 per cent., lymphocytes 22 per cent., monocytes 14 per cent., eosinophils 8 per cent.

Refractory anaemia after treatment with neoarsphenamine; remission.

*Case 30.* An American electrician aged 34 years first complained of weakness three months before the time of his admission to the Rockefeller Institute Hospital. His father had mild diabetes. The patient had had gonorrhoea on numerous occasions, and had treated himself with protargol. At the age of 22 years he had had syphilis and was treated with 24 injections, of which 10 were of neoarsphenamine. At the age of 30 years his Wassermann reaction was found to be positive, and he received eight injections of neoarsphenamine and a similar number of bismuth. The Wassermann reaction was then doubtfully positive. Four months before admission the patient felt unduly

tired and his Wassermann reaction was again found to be positive. An injection of neoarsphenamine was followed by headache, fever, and sub-sternal pain, and the patient had to stay in bed for three days. In spite of the occurrence of further reactions he was given about eight more injections, during which time he became noticeably pale. He was found to be anaemic, and was given two transfusions. On his return home, three weeks before admission, he was given a further dose of neoarsphenamine. This made him 'deathly sick' and he was again admitted to a hospital and given two further transfusions. Bleeding from the gums and cutaneous purpura appeared.

*Physical examination.* Temperature 98.6° F., pulse 82. Well developed and well nourished. Pale and apprehensive. Tongue normal; some blood clot on the gums. No retinal or skin haemorrhages. Chest and heart normal. No enlargement of liver, spleen, or lymphatic glands. Pupils react sluggishly to light. No other neurological abnormality.

*Laboratory investigations.* Blood count on admission (after transfusion), red cells 1,600,000 per c.mm., haemoglobin 30 per cent., colour index 0.9, mean corpuscular volume 81 cub.  $\mu$ , reticulocytes 0.2 per cent., platelets 48,000 per c.mm., white cells 1,150 per c.mm.; differential count, polymorphonuclear leucocytes 25 per cent., lymphocytes 65 per cent., monocytes 10 per cent. Film shows occasional myelocytes and nucleated red cells. Test meal, free hydrochloric acid present. Icterus index 4.

*Sternal puncture.* Fluid contained 21,500 nucleated cells per c.mm. No megakaryocytes seen. Differential count, polymorphonuclear leucocytes, active 40 per cent., non-segmented 16 per cent., myeloblasts 1 per cent., myelocytes 24 per cent., lymphocytes 5 per cent., normoblasts 7 per cent., erythroblasts 7 per cent.

*Further course.* The patient remained afebrile for most of his time in hospital, and he was discharged after three weeks. Two months later his blood count was, red cells 3,100,000 per c.mm., haemoglobin 85 per cent., colour index 1.4, mean corpuscular volume 109 cub.  $\mu$ , white cells 4,550 per c.mm.; differential count, polymorphonuclear leucocytes 54 per cent., lymphocytes 34 per cent., monocytes 12 per cent.

Chronic granulopenia and anaemia in a radiologist. Hypercellular, immature, predominantly erythropoietic marrow. Clinical and postmortem evidence of liver damage.

*Case 31.* An American therapeutic radiologist complained of undue fatigue three or four months before admission to the Rockefeller Institute Hospital. His mother died of cancer of the stomach, and his father was murdered. Of eight siblings three died in infancy, one brother and one sister were said to have hypopituitarism and obesity of the girdle type, and another brother had had a thyroidectomy for adenomatous goitre with hyperthyroidism. The patient suffered from asthma and hay-fever in youth and typhoid fever at eight years old. At 19 years he was thought to have pulmonary tuberculosis, but made a good recovery. At 26 years this condition became active again and he was sent home from service with the British army in France. He had an appendicectomy and cholecystectomy for gall-stones at 30 years, and two operations for exophthalmic goitre at 35 years. He began active work with radium and X-rays at the age of 27 years. After six months work his fingers were affected and he was advised to have his left thumb amputated; at the same time he had a white cell count of 2,000 per c.mm. After two months on other work he was well enough to return to active work

with radium and to a less extent with X-rays, almost up to the time of his admission 18 years later. Nine months before admission he began working with new X-ray apparatus, worked long hours, and apparently became careless about protecting himself from scatter radiations. He began to feel undue weakness, and three months before admission collapsed during a deer-hunt. He was found to have a haemoglobin of 60 per cent. and a white cell count of 2,500 per c.mm. He became progressively worse, and shortly before admission was given two transfusions.

*Physical examination.* Temperature 98.4° F., pulse 106. Well developed and moderately well nourished, pale. Atrophy and shrinkage of the skin, with keratoses (up to 1 cm. diam.) on tips of both thumbs and forefingers. Few petechiae on extremities. Some exophthalmos. Scar of partial thyroidectomy; remaining portion of thyroid enlarged. Brown pigmentation of skin and ankles. Slight atrophy of papillae at margins of tongue. Haemic murmur. No enlargement of spleen, liver, or lymphatic glands. No neurological abnormality.

*Laboratory investigations.* Blood count on admission, red cells 2,200,000 per c.mm., haemoglobin 52 per cent., colour index 1.2, mean corpuscular volume 98 cub.  $\mu$ , reticulocytes 1.2 per cent., platelets 30,000 per c.mm., white cells 1,100 per c.mm.; differential count, polymorphonuclear leucocytes 10 per cent., lymphocytes 55 per cent., atypical mononuclear cells 35 per cent. Icterus index 8. Plasma bilirubin 1.2 mg. per 100 c.c. Urea clearance, first sample, 80 per cent., second sample, 70 per cent. of normal. Average daily urobilinogen output, urine 16.8 mg., stools 23.2 mg.. Basal metabolic rate +14 per cent.

*Further course.* The patient had irregular fever with occasional rigors during the whole time he was under observation. Two blood cultures, taken in febrile periods, were negative. He became slightly jaundiced with an icterus index of 15 and plasma amino-nitrogen of 12.0 mg. per 100 c.c. At the same time he became irrational and drowsy, and it was thought that he had evidence of hepatic insufficiency. Further petechiae and retinal haemorrhages occurred. He died six weeks after admission, about six months after his first symptom of anaemia. His blood count three days before death was, red cells 900,000 per c.mm., haemoglobin 18 per cent., white cells 7,100 per c.mm. In all previous blood counts the white cells were 1,500 per c.mm. Large numbers of normoblasts and some myelocytes were seen in the film.

*Summary of autopsy findings.* Poorly developed and poorly nourished. Slight brownish pigmentation of the skin. Oedema of legs and feet. Multiple small ecchymoses. Few subpericardial haemorrhages. Small healing infarct in lower lobe of right lung, with overlying fibrous and fibrinous pleural adhesions. Spleen (280 gm.) with greyish translucent areas (up to 0.4 cm. diam.) on deep purple red background. Liver (1,620 gm.) with yellowish surface and yellow areas around central veins. No enlargement of lymphatic glands. Sternal, costal, and vertebral marrow greyish, with apparently some fat present.

*Summary of microscopical findings.* Marrow fairly densely hyperplastic with little or no fat. The cellular pattern is not uniform as in leukaemia, but shows some signs of arrangement with conspicuous groups of normoblasts. There are numerous haemocytoblasts and relatively less numerous primary erythroblasts and normoblasts, often with basophil cytoplasm. There are occasional myelocytes, but otherwise no recognizable leucopoietic cells. Numerous megakaryocytes. Liver, severe central necrosis and slight haemosiderosis in portal areas. Spleen, considerable congestion, normal architecture

with prominent lymph follicles and haemosiderosis. Lymphatic gland, small areas of extramedullary erythropoiesis and very occasional myelocytes. A few similar cells in medulla of suprarenal. No sign of leukaemic infiltration.

Anaemia in a clock-dial painter due to the absorption of radioactive substances.

*Case 32.* An American woman first complained of frequent nose bleedings at the age of 23 years,  $2\frac{1}{2}$  years before the time of her admission to the Rockefeller Institute Hospital. She had been employed for nine years as a clock-dial painter, using paint containing radioactive substances, and had been accustomed to point her brushes with her lips. Her family history seemed unimportant. She had been married for five years and she had always eaten a normal diet. Except for influenza in 1918 and a left otorrhoea at times, since childhood she had had no illness. One year before admission she had had severe pain in the right hip, which lasted six weeks, and she did no work for 10 months. Two months before admission she applied for work with another company and was sent for medical examination. She was found to have considerable amounts of radioactive material in her tissues, and to have anaemia and early necrosis of the jaw.

*Physical examination.* Temperature  $100.3^{\circ}$  F., pulse 102. Well developed and well nourished. Moderate pallor. Tongue normal. Cavity  $2.0 \times 0.5$  cm. in position of posterior molars of left lower jaw, lined by yellowish necrotic material. No enlarged lymphatic glands. Systolic murmur at left border of sternum. Liver and spleen not felt. No neurological abnormality.

*Laboratory investigations.* Blood count on admission, red cells 3,400,000 per c.mm., haemoglobin 71 per cent., colour index 1.04, mean corpuscular volume 99 cub.  $\mu$ ., white cells 5,800 per c.mm., differential count, polymorphonuclear leucocytes 69 per cent., lymphocytes 23 per cent., monocytes 4 per cent., eosinophils 2 per cent., myelocytes 2 per cent. Test meal, free hydrochloric acid present after alcohol.

*Biopsy of sternal marrow.* Some densely cellular sheets with little sign of normal architecture; remainder of section fragmented. Cellular areas chiefly erythropoietic, and immature cells with some haemocyto blasts, numerous primary erythroblasts, and relatively fewer normoblasts. Relatively few myelocytes and very few polymorphonuclears. Megakaryocytes in about normal numbers.

*Radiographs of the bones.* There was rarefaction of the heads of both humeri and adjacent portions of the scapulae, areas of softening and of increased density in dorsal spine, both ilia, heads of femora, and skull, and evidence of osteomyelitis of left jaw.

*Further course.* The patient was treated intensively with liver extract, with some temporary improvement in her symptoms and slight improvement in her blood count. After six weeks the count was, red cells 3,600,000 per c.mm., haemoglobin 82 per cent., white cells 4,600 per c.mm. Her blood count 22 months later was, red cells 2,600,000 per c.mm., haemoglobin 50 per cent., white cells 5,700 per c.mm.; differential count, polymorphonuclear leucocytes 73 per cent., lymphocytes 20 per cent., monocytes 5 per cent., myeloblasts 1 per cent., myelocytes 1 per cent. She developed paralysis of the bladder from a transverse myelitis of the cord and died 22 months after she was first seen, about  $4\frac{1}{2}$  years after her first symptoms. An autopsy confirmed that her death was due to 'chronic radium poisoning', but no details are available.

Refractory anaemia apparently associated with the use of a volatile organic insecticide. Marrow at biopsy partly mature and hypercellular, at autopsy similar but less cellular.

*Case 33.* An American bank clerk first complained of bleeding gums at the age of 25 years, 17 months before the time of his admission to the Rockefeller Institute Hospital. His mother was said to have been anaemic and to have bruised easily, but was in good health at the time of the patient's illness. His previous history had been uneventful and his diet had been good. About 22 months before admission he began to spray his pillow at night with a volatile organic insecticide, and did this throughout the summer months to keep mosquitoes away. In the autumn his gums began to bleed and some bleeding continued for most of the winter. He was then free of bleeding for several months. During the following summer he began to use the insecticide again; he liked the smell and stated that he would literally soak his pillow with it each night. After two months, eight months before admission, the bleeding began again. Two nose-bleedings occurred, and four months before admission he was found to be anaemic with a haemoglobin of 40 per cent., and was treated with liver extract. His condition improved and his haemoglobin was said to have increased to 70 per cent. Two months before admission he went to Florida and was able to play golf. While there he was exposed to strong sunlight and he again used the insecticide on his pillow on a number of occasions to keep off gnats. Once more he became worse and was found to have a blood count of red cells 1,500,000 per c.mm. and haemoglobin 35 per cent. He returned to New York, and was admitted to hospital.

*Physical examination.* Temperature 98° F., pulse 88. Well developed and well nourished. Pallor partly obscured by sun tan. Ecchymoses on extremities. Retinal haemorrhages. Haemic murmur. No enlargement of liver, spleen, or lymphatic glands. No neurological abnormality.

*Laboratory investigations.* Blood count on admission, red cells 1,100,000 per c.mm., haemoglobin 26 per cent., colour index 1.2, mean corpuscular volume 107 cub.  $\mu$ ., reticulocytes 5.5 per cent. One nucleated red cell was seen in counting 100 white cells. Platelets 36,000 per c.mm., white cells 3,400 per c.mm.; differential count, polymorphonuclear leucocytes 65 per cent., lymphocytes 30 per cent., monocytes 5 per cent. Films showed anisocytosis, poikilocytosis, and some basophilia. Icterus index 5. Haemolysis of red cells in saline began at 0.42 per cent. and was complete at 0.32 per cent. (control 0.44 to 0.34 per cent.). Test meal, free hydrochloric acid present. Plasma bilirubin 0.4 mg. per 100 c.c. Average daily urobilinogen output, urine 0.6 mg., stools 76 mg. Radiograph of chest showed no abnormality. Wassermann reaction negative. Blood culture negative.

*Biopsy of sternal marrow.* Areas of haemorrhage and areas of quite densely cellular marrow without fat. The cellular areas show partly mature marrow with numerous polymorphonuclears and normoblasts. There are a few haemocyto blasts and numerous primary erythroblasts, and a few myelocytes, a large proportion being eosinophil. The marrow differs from normal in being more densely cellular, showing more erythropoietic than leucopoietic cells, and showing a relative increase of immature erythropoietic forms.

*Further course.* After admission to hospital the patient's condition steadily deteriorated. For some weeks he had fever of a slightly irregular Pel-Ebstein type with bouts of fever up to 103° F. for a few days alternating with periods of subnormal temperature. He developed a necrotic patch on the left tonsil and later on the right tonsil. The red cell count remained about 1,000,000 per c.mm., in spite of 13 blood transfusions of which the effects were quite



temporary, and other treatment. The white cell count decreased to 2,100 per c.mm., and he developed almost complete granulopenia. The icterus index increased terminally to 15. The patient died after a series of generalized convulsions six months after admission, about two years after his first symptoms.

*Summary of autopsy findings.* Well developed and well nourished. Conspicuous freckling. Numerous petechiae. Necrosis of both tonsils. Subcutaneous fat bright yellow. Subpericardial and subpleural haemorrhages. Lungs normal. Spleen (110 gm.) with normal pattern on deep purple cut surface. Liver (1,500 gm.) with uniform deep brown cut surface and some golden-brown pigmentation. Kidneys normal. Lymphatic glands normal. Area of haemorrhage about 4 cm. in diameter in left motor area of brain, communicating with subarachnoid space. Sternal, costal, vertebral, and femoral marrow yellow and apparently fatty.

*Summary of microscopic findings.* Marrow, femoral section almost completely fatty; remaining sections fairly densely cellular with little fat. Few haemocytoblasts, numerous erythroblasts, and normoblasts, almost all with basophil cytoplasm. Relatively fewer myelocytes and polymorphonuclears. No megakaryocytes seen. Section similar to biopsy, but less cellular. Liver, slight haemosiderosis and slight fatty infiltration. Spleen, moderate haemosiderosis and slight erythropoiesis. Lymphatic glands, normal germ centres, pulp strands conspicuous, containing large irregular eosinophil cells, often forming irregular giant cells. There is some haemosiderosis in these areas. No leukaemic infiltration in any organs.

Chronic granulopenia and anaemia, with febrile reactions after the administration of allonal; recurrent infections and stomatitis; atypical pneumonia.

*Case 34.* A married American housewife, 31 years old, had a sudden attack of shivering and fever lasting two to three days, two months before her admission to hospital. Her family history seemed unimportant. For about six years she had suffered from headaches, which were apparently psychogenic in origin, since in spite of numerous investigations no cause for them had ever been discovered. They had improved for a time after an operation for repair of the cervix uteri. For the headaches she had taken large amounts of drugs, of which the precise nature is not known, but they were probably of the pyramidon-barbiturate group. After her first attack of fever she was well for a few days and then suffered from recurrent minor infections, first of the tip of the nose, then of both nostrils with considerable swelling of the face, and then of the lower lip with cervical adenitis. The infections were associated with mild fever. She felt tired and weak, and four weeks before admission went to the seaside where she had a further feverish attack. She was found to have a moderate anaemia and leucopenia. During the two weeks before she was admitted she complained of sore throat and mouth, rectal pain, and unproductive cough. A diagnosis of agranulocytosis was made and she was treated without benefit with 10 injections of pentnucleotide, each of which was followed by a shivering attack.

*Physical examination.* Temperature 99.4° F., pulse 120. Well developed and well nourished. Tongue had yellowish grey coating with large red papillae. Buccal mucous membrane red and rough, with small whitish aphthous ulcerations. Slight cervical adenitis. Chest and heart, no abnormal physical signs. Liver and spleen not felt. No neurological abnormality.

*Laboratory investigations.* Blood count on admission, red cells 2,700,000 per c.mm., haemoglobin 67 per cent., colour index 1.23, mean corpuscular volume

105 cub.  $\mu$ ., white cells 1,800 per c.mm.; differential count, polymorphonuclear leucocytes 36 per cent., lymphocytes 54 per cent., monocytes 8 per cent., eosinophils 2 per cent. Test meal, free hydrochloric acid present. Wassermann reaction negative. *Brucella melitensis* agglutination negative. Widal reaction positive in dilution of 1/80 (patient immunized against typhoid within last few years).

*Sternal biopsy.* Section somewhat fragmented. Sections of marrow of normal architecture, moderately hypercellular and immature, with relative increase of haemocyto blasts and primary erythroblasts and numerous, normoblasts. Very few myelocytes and no polymorphonuclear leucocytes. Few megakaryocytes.

*Further course.* During her stay in hospital the patient had an unproductive cough. Shortly after admission slight dullness, harsh breath sounds, and fine crepitations were noticed at the right apex. Radiographs of the chest suggested an atypical pneumonia. This cleared up in about  $3\frac{1}{2}$  weeks, leaving a faint annular shadow. During this time the patient continued to have a sore mouth with small whitish ulcers, anaemia, leucopenia, granulopenia and slight irregular fever. Shortly after admission, following the administration of 0.32 gm. of allonal she had a shivering attack, rise of pulse, and rise of temperature to 105° F. No treatment was given for her blood condition. Seven weeks after admission her blood count was, red cells 4,200,000 per c.mm., haemoglobin 88 per cent., white cells 3,000 per cent.; differential count, polymorphonuclear leucocytes 44 per cent. At this time she was given deliberately a further dose of 0.32 gm. of allonal. Three hours later she again had a shivering attack and a rise of temperature to 104.6° F. Her absolute polymorphonuclear count, which was 1,544 per c.mm. before the drug was given, increased to 3,850 after four hours, and then decreased to 2,993 after five hours, 2,015 after 11 hours, 1,375 after 21 hours, 1,118 on the second day, and 899 on the third day. The patient was discharged after two months, considerably improved with haemoglobin 87 per cent. and 2,900 white cells per c.mm., and was advised to take no drugs containing barbiturates or pyramidon. After 10 months her blood count was, red cells 4,600,000 per c.mm., haemoglobin 94 per cent., white cells 8,550 per c.mm. During the succeeding five years she has continued to suffer from headaches, for which she has taken codeine, but no aromatic compounds. She has had occasional attacks of sore throat with cervical adenitis, but her blood count has remained within normal limits.

Anaemia possibly associated with poor diet and excessive consumption of acetanilide and barbiturates; hypercellular immature predominantly erythropoietic marrow.

*Case 35.* A married American housewife was first found to be anaemic four years before admission to the Rockefeller Institute Hospital. She had been nervous for as long as she could remember, and had had numerous nervous breakdowns. She had also suffered from indigestion, nausea, headaches, and for 10 years from 'asthma'. Because of her indigestion she had gradually eliminated one food after another from her diet. For many years this had been inadequate, with little or no meat or milk and little fruit. Four years before admission she consulted a doctor because of 'arthritis' and was found to be moderately anaemic. Iron was ineffective, but on treatment with liver extract the haemoglobin increased from 60 to 85 per cent. Two years before admission her haemoglobin was again 60 per cent., but on this occasion treatment with both liver and iron was ineffective, and the anaemia slowly became

more severe. For five to six years, and especially in the two years before admission, she had been taking large amounts of analgesic drugs for her headaches (2 to 6 capsules daily containing acetanilide, and up to 6 gr. of phenobarbitone frequently at night).

*Physical examination.* Temperature 97° F., pulse 100. Nervous, restless, and poorly nourished. Moderate pallor. Dry skin. Tongue normal. No haemorrhages. Chest and heart normal. Liver just felt. No enlargement of spleen or lymphatic glands. No neurological abnormality.

*Laboratory investigations.* Blood count on admission, red cells 1,600,000 per c.mm., haemoglobin 41 per cent., colour index 1.3, mean corpuscular volume 102 cub.  $\mu$ ., platelets 154,000 per c.mm., white cells 4,100 per c.mm.; differential count, polymorphonuclear leucocytes 68 per cent., lymphocytes 16 per cent., monocytes 16 per cent.; 2 normoblasts and 2 erythroblasts seen in counting 100 white cells. Film showed macrocytosis and anisocytosis. Test meal, free hydrochloric acid present. Plasma bilirubin 0.8 mg. per 100 c.c. Average daily urobilinogen output, urine 2.6 mg., stools 94.0 mg. Basal metabolic rate + 25 per cent.

*Further course.* The patient was discharged and treated as an out-patient with liver extract and betaxan. She continued to take nembutal daily and to eat her customary poor diet. Six weeks later she was re-admitted. Her condition deteriorated in spite of two blood transfusions, and she developed a rectal haemorrhage, thrombosis of the right antecubital vein, and rales at the bases of both lungs. She died one month after her second admission, just over four years after she was first found to be anaemic. Her blood count four days before death was red cells 2,100,000 per c.mm., haemoglobin 44 per cent., colour index 1.0, white cells 4,300 per c.mm.; differential count, polymorphonuclear leucocytes 46 per cent., basophils 2 per cent., lymphocytes 30 per cent., monocytes 22 per cent.

*Summary of autopsy findings.* Well developed, but poorly nourished. Oedema of hands and feet. Slight pigmentation of skin. Superficial skin ulcerations over sacrum. Early bronchopneumonia of middle lobe of right lung. Liver (1,780 gm.) slightly enlarged with red portal areas and yellow centres of lobules on cut surface. Spleen (200 gm.) with normal markings. No haemorrhages. No enlargement of lymphatic glands. Sternal, costal, femoral, and vertebral marrow uniformly yellow and apparently aplastic.

*Summary of microscopical findings.* Sternal, costal, and vertebral marrow diffusely and slightly hypercellular. Fat and normal architecture in femoral sections. There are fairly numerous haemocytoblasts, very numerous primary erythroblasts, and relatively few normoblasts, almost all with more or less scanty basophil cytoplasm. Relatively few myelocytes and almost no polymorphonuclears. Megakaryocytes in about normal numbers. Slight haemosiderosis. Liver, severe central necrosis and moderate haemosiderosis. Spleen, considerable haemosiderin, some extramedullary erythropoiesis with fairly numerous primary erythroblasts, and a few normoblasts. Suprarenal, slight extramedullary erythropoiesis. Lung, early bronchopneumonia.

Refractory anaemia with severe granulopenia; predominantly erythropoietic marrow; possible association with excessive medication and restricted diet.

*Case 36.* An American police court officer first complained of weakness and pallor at the age of 60 years, nine weeks before admission to the Rockefeller Institute Hospital. His family history did not appear significant and he had had no ill health of importance until five years before the onset of

his symptoms, when he began to suffer from flatulent dyspepsia and constipation. His haemoglobin was normal at this time. On account of this dyspepsia he was placed on a diet which contained large amounts of starch, but almost no fruit. For several years he had taken large amounts of a variety of drugs, but he was quite unable to recall what the medicines were. Six weeks before admission he was found to be anaemic, and was treated with liver extract at first by mouth and later by injection, without benefit.

*Physical examination.* Temperature 98·6° F., pulse 90. Well developed and well nourished. Pale but not jaundiced. Tongue normal. Chest and heart normal. No enlargement of liver, spleen, or lymphatic glands. No neurological abnormality.

*Laboratory investigations.* Blood count on admission, red cells 1,600,000 per c.mm., haemoglobin 47 per cent., colour index 1·5, mean corpuscular volume 125 cub.  $\mu$ , reticulocytes 1·7 per cent., platelets 148,000 per c.mm., white cells 2,450 per c.mm.; differential count, polymorphonuclear leucocytes 4 per cent., lymphocytes 84 per cent., lymphoblasts 12 per cent. Icterus index 5. Test meal, complete achlorhydria after histamine.

*Biopsy of sternal marrow.* Somewhat patchy, but on the whole of about normal cellularity. The cellular areas consist for the most part of densely packed small cells with basophil nuclei and almost no cytoplasm. Amongst them there are groups of normoblasts, occasional eosinophil myelocytes, and some primary erythroblasts. The marrow is therefore an immature one, probably predominantly erythropoietic in type, of about normal cellularity.

*Further course.* This patient was at first thought to have pernicious anaemia, but he failed to show any response to injections of liver extract, and the bone marrow sections when completed failed to confirm that diagnosis. In his third week in hospital he was given a dose of 1·2 gm. of pyramidon for a study of his ability to conjugate this compound. On the following day his temperature, which up to that time had been practically normal, rose suddenly to 102° F. and remained elevated for four days. At the same time he complained of soreness and tenderness of the gums. The gum margins appeared white, and exudate appeared between them and the teeth. His throat became red, injected, and oedematous, with a few small vesicles on the mucous membrane. The possibility of coincidence cannot be ruled out, but it seems most probable that these symptoms were an abnormal response to pyramidon. The patient went home four weeks after admission. Subsequent attempts to trace him elicited the information that he was dead, though the date of his death is not known.

*Effects of sunlight, ultraviolet light, and X-irradiation.* Several patients had been recommended, when they were first found to be anaemic, to take a sea voyage or a visit to Florida. In six cases the patients appeared to be worse after exposing themselves to sunlight; in one of them purpura and bleeding from the gums began suddenly after exposure to sunlight during a voyage to the West Indies, and in another exposure to sunlight on several occasions produced nausea, fever, and headaches. Three patients with cellular marrow were treated with X-irradiation in the belief that they were suffering from leukaemia, and a therapeutic sterilization with X-irradiation was attempted in two, in one of whom haemolytic episodes appeared to coincide with menstruation. Of these patients, two stated that they felt worse during the time they were receiving irradiation and better when it was abandoned.

A third received only three treatments with a very small dose, each of which was followed by nausea and vomiting; at the same time the red cell count, white cell count, and haemoglobin all decreased sharply. In a fourth patient one of four projected treatments was given to produce a therapeutic sterilization. Six days after the first treatment the patient was found to have a white cell count of 650 per c.mm., slight jaundice and an icterus index of 20. Further irradiation was therefore abandoned. In the fifth case the patient stated that she felt worse after two deep X-ray treatments to the pelvis, and her menorrhagia was not improved. In one patient treatment with Alpine light was followed by nausea and palpitation, and another patient felt better when treatment of this kind was abandoned. There is perhaps enough evidence here to suggest that these patients are in some way sensitive to light and X-rays. The history of reactions after exposure to natural sunlight raises the question whether these patients, who often excrete increased amounts of coproporphyrin I, may have had a light sensitivity due to the presence of porphyrin in the skin and analogous to that seen in porphyria.

### *Case Reports*

Refractory anaemia with change in type of marrow from partly mature cellular to immature cellular, possibly associated with taking of atophan. Deterioration, apparently following exposure to strong sunlight.

*Case 37.* An American housewife first had an attack of fever with rigors and was found to be anaemic at the age of 36 years, eight months before admission to the Rockefeller Institute Hospital. Her previous history and family history appeared unimportant, except for the fact that one of her children developed diabetes at the age of three years. Menstruation was regular and normal before her illness, and she had always eaten a good diet. Eleven months before admission she took 24 tablets of atophan in about six weeks for a pain in the right shoulder. Shortly after this she developed a feeling of tightness in her chest followed by fever with rigors. She was admitted to a hospital and found to have a moderate anaemia with haemoglobin 52 per cent., severe leucopenia with white cells 850 per c.mm., and severe thrombocytopenia. During the next seven months she had three attacks of fever and rigors, each lasting some weeks, with intervals of normal temperature. In spite of the most complete investigation in two different hospitals, no cause for the fever, other than anaemia, was found. She was treated with large amounts of liver extract and given three blood transfusions.

*Physical examination.* Temperature 102° F., pulse 100. Well developed and well nourished. Pale, without jaundice. No retinal or skin haemorrhages. Tongue normal. Haemic murmur. Chest normal. No enlargement of liver, spleen, or lymphatic glands. No neurological abnormality.

*Laboratory investigations.* Blood count on admission, red cells 3,000,000 per c.mm., haemoglobin 65 per cent., colour index 1.1, mean corpuscular volume 98 cub.  $\mu$ , white cells 1,500 per c.mm.; differential count, polymorphonuclear leucocytes 40 per cent., lymphocytes 55 per cent., monocytes 2 per cent., eosinophils 3 per cent. Plasma bilirubin 0.4 mg. per 100 c.c. Barium meal and radiograph of chest normal.

*Sternal marrow biopsy.* (Section from New York Hospital taken four months before admission.) Section shows marrow of normal or slightly increased cellularity, containing numerous haemocyto blasts, erythroblasts,

and normoblasts; less numerous myelocytes, mostly eosinophil, and few polymorphonuclears. No megakaryocytes seen. Section five months after admission consisted chiefly of red cells and pink structureless material, with islands of marrow of about normal cellularity. The cell picture was very similar to that of the previous biopsy except that it was much less dense and there was a relative increase of more immature forms. No megakaryocytes were seen.

*Further course.* The patient was in hospital on three occasions and for eight months in all. During this time she persistently had irregular fever with occasional rises to 104° or 105° F. and rigors. About six months after admission, her spleen became palpable and from this time she had some bleeding from the gums, retinal haemorrhages, and petechiae. Up till this time her red cells and haemoglobin declined only very slowly and no transfusions were needed. Eight months after admission, while on a short vacation, the patient sat in the sun and was severely burned on the arms and legs; for days later her white count, for a considerable time previous about 1,000 per c.mm., was 200 per c.mm. Ten days after this episode she was readmitted with red cells 700,000 per c.mm., haemoglobin 12 per cent., and white cells 350 per c.mm. Thereafter her condition steadily deteriorated in spite of seven blood transfusions and other treatment. She died one year after her first admission, 19 months after her first symptom.

*Summary of autopsy findings.* Well nourished. No haemorrhages or jaundice. Small areas of haemorrhage in lungs. Heart normal. Liver (965 gm.) with irregular red and yellow areas on outer surface and cut surface, showing areas apparently of necrosis and regeneration. Spleen (245 gm.) with purple cut surface and prominent follicles. Small cysts in ovaries. Femoral, vertebral, sternal, and costal marrow appears cellular and fatty.

*Summary of microscopical findings.* Marrow sparsely but evenly cellular with normal architecture and about normal number of fat cells. The cells are mostly haemocytoblasts, erythroblasts, and some basophil normoblasts. A few eosinophil myelocytes; no megakaryocytes seen. Liver, severe fatty degeneration with some central necrosis and slight haemosiderosis. Spleen, considerable haemosiderosis; few red cells in pulp; slight erythropoiesis of the same type as that in the marrow. Lymphatic glands, slight erythropoiesis in some sections.

*Pigmentation of the skin and haemochromatosis in refractory anaemia.* Well marked pigmentation of the skin was seen in three cases in this series and was associated with fibrosis of the liver, with the presence of pigment in the pancreas and other organs, and in one case with diabetes. Lesser degrees of pigmentation of the skin were noticed in nine other cases. In all these cases the marrow at biopsy was of the partly mature cellular type.

When most conspicuous the pigmentation appeared as a mottled greyish brown or bronze discoloration; in other cases it resembled unusually conspicuous freckling. It was distributed mainly in areas exposed to light; that is, on the hands, forearms, neck, and face, and only slightly elsewhere on the body. The association of refractory anaemia with pigmentation of this type was described by Kark (1937) in a patient with long-standing anaemia, who received 290 blood transfusions in nine years. A sternal biopsy early in the course of the illness showed a cellular marrow. Of our three patients with haemochromatosis, two had not received an exceptional number of trans-

fusions, and one had received 54 over a period of nine years. The occurrence of haemochromatosis as a complication of anaemia of this type raises interesting problems in itself and in connexion with the aetiology of idiopathic haemochromatosis, but these matters cannot be discussed here.

### *Case Reports*

Refractory anaemia with hypercellular partly mature marrow; haemochromatosis as a complication.

*Case 38.* An American lumberman, aged 68 years, first complained of undue fatigue seven months before admission to the Rockefeller Institute Hospital. His family history appeared unimportant. His diet had always been good and his weight was increasing. Seven months before admission he was found to be severely anaemic with a red count of 1,500,000 cells per c.mm. Treatment with liver extract by mouth and injection, copper, iron, and a yeast concentrate failed to produce any improvement in his condition. He had no haemorrhage or other symptoms except dyspnoea and swelling of the feet.

*Physical examination.* Temperature 100.2° F., pulse 100. Well developed and slightly obese. Skin pale with subicteric tint. No skin or retinal haemorrhages. Right ear-drum perforated. Some atrophy of papillae at edges of tongue. Chest normal. Haemic murmur. No undue thickening of arteries. Blood pressure 112/70. No enlargement of liver, spleen, or lymphatic glands. Perception of vibration greater in right leg than left. No other neurological abnormality.

*Laboratory investigations.* Blood count on admission, red cells 1,300,000 per c.mm., haemoglobin 35 per cent., colour index 1.3, mean corpuscular volume 104 cub.  $\mu$ , reticulocytes 1.4 per cent., white cells 4,350 per c.mm.; differential count, polymorphonuclear leucocytes 55 per cent., lymphocytes 33 per cent., monocytes 4 per cent., eosinophils 4 per cent., myelocytes 4 per cent. Test meal, free hydrochloric acid present after histamine. Barium meal and barium enema, no abnormality discovered. Radiograph of chest, no abnormality discovered.

*Biopsy of sternal marrow.* Marrow hypercellular with normal architecture. There are numerous polymorphonuclear leucocytes and normoblasts; a few haemocyto blasts, myelocytes, and primary erythroblasts. Megakaryocytes present, but reduced in numbers. The marrow therefore appears partly mature with a slight preponderance of erythropoietic cells.

*Further course.* Except for one interval of seven weeks, the patient remained in hospital until his death 16 months later. His temperature remained about normal with occasional rises to 100° F. He was given 12 blood transfusions with no more than temporary benefit, and numerous other treatments including intravenous liver extract, whole embryonic extract, intramuscular haemoglobin injections, whole liver, beef intestine, vitamin digest, vegex, cystine, and glycine by mouth, and beef bile by stomach tube. Shortly after admission he had a severe coryza, and after this several cultures from the left sphenoidal sinus grew a haemolytic staphylococcus aureus. As no other abnormality in the sinuses could be found, operative treatment on the sinus was not undertaken. After some months brown pigmentation of the skin was noticed, particularly on the backs of the hands and the neck. Later there was a wide-spread bronze pigmentation of the skin. In between transfusions the patient complained of dyspnoea, and at times had some oedema of the legs and ascites which disappeared with rest in bed. No haemorrhages occurred. About two weeks before death the patient developed increasingly

severe jaundice, followed by attacks of hiccups. He died 16 months after his first admission, 23 months after his first symptom. Throughout this time he remained severely anaemic with red count between 1,000,000 and 2,000,000 per c.mm., moderate macrocytosis, white count between 2,000 and 5,000 per c.mm., and a differential count within normal limits except for the appearance of occasional myelocytes.

*Summary of autopsy findings.* Body well developed and well nourished. Skin has brassy colour and mottled brown pigmentation of neck and arms. Bright yellow subcutaneous fat. Mesenteric and thoracic lymphatic glands slightly enlarged; on section the sinusoids are marked out by golden-brown pigment. Bronchopneumonia of right lower lobe. Spleen (130 gm.) with brownish-purple cut surface and normal markings. Liver (1,840 gm.) with abundant golden-brown pigment around portal areas. Kidneys brownish in colour. Pancreas contains considerable amounts of fat; pancreatic tissue deep brown in colour. Golden-brown pigment in cortex of suprarenals and in choroid plexus. Sternal, costal, vertebral, and femoral marrow deep red and apparently cellular.

*Summary of microscopical findings.* All sections of marrow hypercellular, with some fat spaces and normal architecture. There are some haemocyto blasts, numerous primary erythroblasts, and some normoblasts, mostly with basophil cytoplasm. Few myelocytes, mostly eosinophil, and very few polymorphonuclears. Megakaryocytes little reduced. Considerable haemosiderosis. Liver, multilobular fibrosis with severe haemosiderosis. Fibrosis and haemosiderosis of pancreas. Spleen shows foci of extramedullary haemopoiesis with megakaryocytes and normoblasts. Lymphatic glands, follicles appear normal; pulp contains large amounts of haemosiderin. Pigment in basal layers of epithelium of skin and in choroid plexus. Right lung, bronchopneumonia.

Refractory anaemia with change in type of marrow from partly mature cellular to immature cellular, and haemochromatosis as a terminal complication.

*Case 39.* An American schoolboy was admitted to the Rockefeller Institute Hospital at the age of 18 years. He first had severe nose-bleeding from the age of five to seven years. He was then well until at 10 years he had swollen bleeding gums and numerous ecchymoses. At 12 years a tonsillectomy was followed by severe haemorrhage, and bleeding occurred after tooth extractions. From this time he began to notice weakness and pallor. The next year he had pneumonia, and when this had subsided he was given a blood transfusion and a splenectomy was done. The spleen was slightly enlarged, but was reported to have been of normal structure. In the five years after this operation the patient had 38 blood transfusions; after each one he felt well and strong for a short time, but soon became weak and dyspnoeic. He had no bleeding after the splenectomy till two weeks before admission, when slight oozing from the gums appeared. His family history appeared unimportant, his diet had been good, and there was no history of exposure to toxic substances.

*Physical examination.* Temperature 100.2° F., pulse 104. Well developed and well nourished, but very pale. Slight brown pigmentation of arms. Gums retracted, no bleeding. No retinal or cutaneous haemorrhages. Haemic murmur. No enlargement of liver or lymphatic glands. No neurological abnormality.

*Laboratory investigations.* Blood count on admission, red cells 1,300,000



per c.mm., haemoglobin 26 per cent., colour index 1.0, mean corpuscular volume 86 cub.  $\mu$ , reticulocytes 0.4 per cent., platelets 60,000 per c.mm., white cells 3,850 per c.mm.; differential count, polymorphonuclear leucocytes 36 per cent., lymphocytes 60 per cent., monocytes 4 per cent. Films showed anisocytosis, macrocytosis, little poikilocytosis. Icterus index 2. Test meal, free hydrochloric acid present after alcohol.

*Sternal marrow biopsy.* Section shows large sheets of densely cellular tissue with almost no fat-cells, separated by large areas of pink fibrillar material. The cellular areas contain haemocytoblasts, erythroblasts, normoblasts, myelocytes, and quite numerous polymorphonuclears. The marrow is therefore a partly mature cellular one. A few megakaryocytes are present.

*Further course.* On his first admission the patient was in hospital for three months. He had irregular fever with frequent rigors, some of which followed liver extract or transfusions; for others there was no evident cause. His condition remained essentially unchanged, with persistent anaemia and leucopenia, reticulocytes between 0.5 and 1.6 per cent., and mean corpuscular volume up to 110 cub.  $\mu$ . Occasional myelocytes and nucleated red cells were seen in stained films. One year later a mottled dusky brown pigmentation of the skin was first noticed. In several subsequent admissions there was fever with further rigors, and bleeding continued. Blood cultures were repeatedly negative. No treatment had any effect on the blood picture, and benefit from transfusions lasted only a short time. Three years after his first admission he complained of dryness of the mouth, thirst, polyuria, and a rapid loss of weight. He was re-admitted and found to be drowsy and dehydrated. The dusky brown pigmentation of his skin was deeper in colour, especially over the hands and arms. At this time there was no bleeding and no enlargement of the liver or lymphatic glands. Sugar was present in the urine, and the blood-sugar was 50 mg. per 100 c.c. The patient was treated with large doses of insulin, but two days later developed above and behind the right ear an abscess which was incised. He developed high fever, and two blood cultures grew *Bacillus coli*. His condition deteriorated and he died three weeks after admission, three and a half years after he was first seen. His blood count one week before death was, red cells 1,400,000 per c.mm., haemoglobin 30 per cent., colour index 1.07, white cells 6,600 per c.mm.

*Summary of autopsy findings.* Well-developed emaciated youth. Diffuse brown pigmentation of skin. Draining abscess behind right ear. Right pleural cavity obliterated by fibrous adhesions. Oedema of lungs and early bronchopneumonia. Myocardium pale and light brown in colour. Liver enlarged and chocolate-brown in colour. Pancreas firm and deep brown in colour. Right kidney enlarged with multiple abscesses (up to 6 mm. diam.). Femoral, sternal, and vertebral marrow fatty, with some cellular areas, and brown in colour.

*Summary of microscopical findings.* Sternal, vertebral, and costal marrow diffusely hypercellular with little fat; femoral marrow moderately cellular, with fat and normal architecture. There are numerous haemocytoblasts, often in small groups, numerous primary erythroblasts, and a few basophil normoblasts; relatively few eosinophil and neutrophil myelocytes; no megakaryocytes seen; there are groups of lymphocytes forming typical nodes; very slight haemosiderosis. Liver, extremely conspicuous haemosiderosis with early portal fibrosis. Pancreas, considerable haemosiderosis with strands of early fibrosis. Lymphatic glands, severe haemosiderosis. Slight haemosiderosis of suprarenals and cardiac muscle. Right kidney, small

abscess containing small round cells and bacteria. In adjacent areas there is some infiltration, with cells closely resembling those in the marrow, including occasional basophil normoblasts; there is therefore apparently extra-medullary haemopoiesis. A few similar areas in left kidney.

### *Macrocytosis in Refractory Anaemia*

The average mean corpuscular volume in 53 cases was 98 cub.  $\mu$ , and the figures varied from 75 to 120 cub.  $\mu$ . In 19 cases the mean corpuscular volume was 95 cub.  $\mu$  or less, and in 30 cases it was above 95 cub.  $\mu$ . In nine cases with hypoplastic marrow the average mean corpuscular volume was 96 cub.  $\mu$ , with limits from 75 to 107 cub.  $\mu$ . In 11 cases with immature cellular marrow the average mean corpuscular volume was 102 cub.  $\mu$ , with limits from 94 to 118 cub.  $\mu$ . In 28 cases with partly mature cellular marrow the average mean corpuscular volume was 96 cub.  $\mu$ , with limits from 80 to 120 cub.  $\mu$ . In four cases with sclerotic marrow the average mean corpuscular volume was 101 cub.  $\mu$ , with limits from 94 to 106 cub.  $\mu$ . Slight macrocytosis is therefore frequently found in refractory anaemia, and there does not appear to be any significant difference between the four groups in this respect. No Price-Jones curves have been drawn, but as far as it is possible to judge from the appearance of cells in films, the macrocytosis is associated with relatively little anisocytosis and very little poikilocytosis. It appears therefore to be unlike the variety found in pernicious anaemia, but like that seen in liver disease and in the variety of anaemia associated with myxoedema. When a remission sets in, the degree of macrocytosis often increases considerably.

### *Liver Function in Refractory Anaemia*

Two liver function tests were employed. In the bilirubin excretion test of Harrop and Barron (1931) 1 mg. of crystalline bilirubin per kilogram of body-weight is injected intravenously. The amount of bilirubin retained in the plasma after four hours is estimated by a colorimetric method, and a retention of more than 5 per cent. of the injected bilirubin after four hours is regarded as abnormal.

The sodium benzoate conversion test of Quick (1933) was also employed, in the hope that it might serve as a measure of the detoxifying power of the liver. Sodium benzoate is converted by the liver to hippuric acid and excreted as such. The hippuric acid excreted in the urine during four hours is measured and expressed in terms of benzoic acid. According to Quick, if 5.9 gm. of sodium benzoate is taken by mouth, the equivalent of 3.0 gm. of benzoic acid should be excreted within four hours. Failure to excrete this amount is regarded as indicating liver insufficiency, provided that intestinal absorption and renal function are normal.

A third test of liver function, and possibly the best available, is provided by the rate of urobilinogen excretion in the urine and faeces. An increase

in the amount excreted in the urine, without a corresponding increase in that excreted in the faeces, is thought to indicate an impairment of liver function (Watson, 1937, 1938).

The results of liver function tests in some of the patients in this series have already been reported by Barker (1938). The bilirubin retention test was performed on 26 patients, and values above normal (from 6.3 to 21 per cent.) were present in 12. The plasma bilirubin before the injection of bilirubin in these 26 patients varied from 0.3 to 1.9 mg. per 100 c.c. The sodium benzoate conversion test was performed on 24 patients, and values below normal (2.6 to 0.0 gm. in four hours) were present in eight. In 21 patients both tests were done and both were abnormal in six. The normal amount of urobilinogen excreted in the urine daily according to Watson (1937) is 0.5 to 2.0 mg. Values above normal (2.7 to 23.2 mg.) were present in nine of 25 patients, without a proportionate increase in the excretion of urobilinogen in the faeces. In four of these the bilirubin excretion test and the sodium benzoate conversion test were also abnormal.

According to the results of these tests there was good evidence of impairment of hepatic function (abnormality in all three tests) in four patients, and in 14 out of 26 patients one or other of the three tests was abnormal. If these results are accepted as evidence of liver insufficiency, their interpretation is still difficult, since, as in the case of the morphological changes seen in the liver, it is possible that the impairment of liver function is secondary to the anaemia. Certain observations suggest, however, that the impaired liver function in these patients is not merely secondary to the anaemia. Firstly, in the nine patients with abnormal results in two of the three liver function tests, the average haemoglobin was 42 per cent.; and in four of them, including two of the four in which all three tests were abnormal, the haemoglobin was above 50 per cent. If the liver insufficiency were simply secondary to the anaemia, it might be expected that its occurrence would show some relation to the severity of the anaemia. Secondly, in six cases of moderate anaemia attributed to benzol poisoning studied in this department and reported in full by Erf and Rhoads (1939), these tests were performed when the patients were first seen and again when their haemoglobin had returned to normal. In two cases the liver function appeared to have improved in the interval, in two there was no change, and in two the results were more abnormal on the second occasion than on the first. This result is surprising and lacks confirmation, but as has been discussed in the section dealing with remissions, it is consistent with the fact that many patients in remission show a low red cell count, a high colour index, and macrocytosis, a haematological picture often seen in liver disease.

Taking into account the morbid anatomical changes in the liver already described and the results of liver function tests discussed in this section, it seems possible that damage to the liver in varying degrees is an important factor in the aetiology of refractory anaemia, and one which merits further investigation.

*Pigment Metabolism in Refractory Anaemia*

In recent years understanding of the nature of certain disorders of the blood has been considerably advanced by studies of pigment metabolism in health and disease, and particularly by the application of quantitative methods to the estimation of the excretion of bile pigments and porphyrins. These methods appear to provide in certain conditions a means of estimating independently the rate of destruction and construction of haemoglobin, and thus of approaching the vexed question of the part played by haemolysis in different blood disorders.

(a) The excretion of porphyrins. To make the findings in refractory anaemia intelligible to those unfamiliar with this field, a bare outline of some recent developments in the study of porphyrin metabolism will be given. Reference should be made to the original papers, to Watson's contribution to the subject of the pyrrol pigments in Downey's *Handbook of Haematology* (1938), Watson's article in the *Wisconsin Symposium* (1939), and to a review by Dobriner and Rhoads (1940).

Haemoglobin consists of a protein, globin, in combination with iron and a porphyrin. All porphyrins are constructed with four pyrrol nuclei, united by four methene bridges to form a porphyrin ring, with eight substituting groups at the periphery of the ring. Four of these are always methyl groups. The nature of the remaining substituting groups determines the variety of porphyrin (i.e. coproporphyrin, protoporphyrin, &c.), and the positions of the substituting groups determine the isomeric type of the particular variety of porphyrin. The simplest variety of porphyrin, in which the substituting groups consist only of four ethyl and four methyl groups, is etioporphyrin, of which four isomeric types only are possible. All porphyrins of biological importance can be divided into four isomeric types, according to the type of etioporphyrin from which they could theoretically be derived.

Biologically important porphyrins, with the exception of uroporphyrins, are obtained from biological materials by ether extraction. When roughly purified, the different varieties can be separated from ether by the use of their selective solubilities in different concentrations of hydrochloric acid (Dobriner, 1936), and when purified, converted to their methyl esters and crystallized; the variety and isomeric type can be determined by the melting-point of their crystalline esters. These methods of identification and accurate quantitative estimation of porphyrins are too complicated for ordinary clinical use. Simpler methods for the roughly quantitative determination of the amount of porphyrin excreted in the urine have been described by Rimington (1938) and Dobriner and Rhoads (1938 *b*). They are well adapted for clinical purposes, but do not determine the isomeric type of the porphyrin present. In nature, porphyrins of only two of the four possible isomeric types have so far been discovered. Haemoglobin, chlorophyll, cytochrome C, and other respiratory pigments contain a type III porphyrin, while normal animals and normal human subjects excrete in the urine and

faeces small amounts of a type I coproporphyrin. This discrepancy between the type of porphyrin present in respiratory pigments and that excreted in the urine was referred to by Fischer as the dualism of the porphyrins.

On theoretical grounds it is inconceivable that the type I porphyrin in the excreta can be derived from the breakdown of the type III porphyrin of the respiratory pigments, for such a conversion could not occur without a breakdown of the porphyrin ring into simple pyrroles and a resynthesis of the pyrroles to form a type I porphyrin. Under normal conditions the porphyrin containing respiratory pigments break down to bile pigments (Lemberg, 1935) without going through a stage in which free porphyrins occur. These facts led Dobriner, Localio, and Strain (1936) in America and Rimington (1936) in South Africa independently and practically simultaneously to suggest that the small amounts of type I porphyrin present in the excreta are formed as a by-product in the natural synthesis of the large amounts of type III porphyrin necessary for the production of the respiratory pigments. Dobriner particularly (Dobriner, 1936; Dobriner, Strain, Localio, Keutmann, and Stephens, 1937; Dobriner and Rhoads, 1938*a*) has developed the hypothesis that in disorders of the blood, where the relationship between the amounts of these two types of porphyrin is not disturbed, the rate of excretion of coproporphyrin I in the urine and faeces gives an indication of the rate of production of haemoglobin. The evidence for this hypothesis is that normal animals and normal human subjects with a constant rate of haemoglobin production excrete coproporphyrin I at an approximately uniform rate. In dogs the removal of 1,000 c.c. of blood is followed by an increase in the amount of coproporphyrin I excreted, the increase occurring at the same time as the reticulocyte response and the beginning of haemoglobin regeneration (Dobriner and Rhoads, 1938*a*). There is evidence to suggest that the blue staining material in reticulocytes is in fact protoporphyrin type III (Watson and Clarke, 1937; Grotepass, 1937). In dogs rendered anaemic by the injection of phenylhydrazine, there is an immediate increase in the total amount of bile pigments excreted corresponding to the haemolysis, and a little later there is an increase in the total excretion of coproporphyrin I corresponding to the beginning of haemoglobin regeneration (Dobriner, 1937*b*). The same sequence was observed in a patient with polycythaemia treated with phenylhydrazine (Dobriner, Strain, Localio, Keutmann, and Stephens, 1937). Further, in the developing chick embryo coproporphyrin I appears almost coincidently with the type III porphyrins (Schönheyder, 1938).

Increased coproporphyrin I excretion, in the absence of any evidence of a qualitative disturbance in the metabolism of pigments, has been demonstrated in three blood disorders, haemolytic jaundice (Watson, 1935; Dobriner, 1937*a*), polycythaemia (Dobriner, 1937*a*) and pernicious anaemia<sup>2</sup> (Watson,

<sup>2</sup> It is not possible to discuss here the nature of pernicious anaemia; we believe, however, that it would be difficult for anyone, approaching the evidence now available with no preformed conception of the nature of this disorder, to avoid the conclusion

1935; Dobriner and Rhoads, 1938 *a*). These findings appear to indicate an increased rate of haemoglobin production without a serious qualitative disturbance in pigment metabolism. In certain other disorders coproporphyrin III is excreted in the urine and faeces, probably always in addition to small amounts of coproporphyrin I, and this appears to indicate a qualitative disturbance either in the synthesis or in the breakdown of haemoglobin or other respiratory pigments. The main disorders in which excretion of coproporphyrin III has been demonstrated are lead poisoning (Grotepass, 1932; Watson, 1936 *b*), salvarsan poisoning (Schreus and Carrié, 1933), sulphanilamide poisoning (Rimington, 1939), diseases of the liver (Dobriner, 1936, 1937 *a*; Vigliani and Libowitzky, 1937), and refractory anaemias, both those due to known toxic substances and those occurring apparently idiopathically (Dobriner, Rhoads, and Hummel, 1938).

Dobriner determined the average daily coproporphyrin and urobilinogen excretion in six patients with refractory anaemia over periods of 36 to 108 days. Five of these are patients included in this report. In these six patients the total urobilinogen excreted was within normal limits in three, doubtfully increased in one, and definitely increased above normal limits in two. The total excretion of coproporphyrin was below normal in two cases, moderately increased in two, and considerably increased in two. There appeared to be no correlation between the type of bone marrow present and the amount of coproporphyrin excreted. In four patients there was a mixed excretion of coproporphyrins I and III; in the remaining two coproporphyrin I was identified and the presence of coproporphyrin III was suggested but not proven. Approximately estimated, the proportion of coproporphyrin III to coproporphyrin I excreted daily in the urine and faeces was 1/5 or less. The simultaneous excretion of coproporphyrin of types I and III in four cases of refractory anaemia indicates that the pigment metabolism in this condition differs from that present in pernicious anaemia and haemolytic jaundice, but is similar to that found in certain diseases of the liver, including haemochromatosis, and to that in lead, sulphanilamide, and salvarsan poisoning. In this connexion certain claims by Thomas (1938) are of interest. He states that if normal rats are injected with haemoglobin this is broken down and excreted as bile pigments without any increase in the excretion of porphyrin. If, however, the rats are first poisoned with phosphorus, part of the haemoglobin is broken down in an abnormal manner and a protoporphyrin, by inference protoporphyrin III, appears in the excreta.

The study of the porphyrins excreted in refractory anaemias indicates, therefore, that the disturbance of pigment metabolism in these conditions is of the type seen in certain disorders of the liver and in certain forms of exogenous poisoning. According to Thomas the same disturbance can be produced experimentally in animals by the administration of phosphorus, a powerful liver poison.

that an increased rate of haemolysis, and therefore of erythropoiesis, is present in the disease (Dock, 1936).

(b) The excretion of bile pigments. Bilirubin enters the intestine from the bile ducts, and is broken down during its passage through the intestine. The exact steps by which this breakdown occurs are not known, but the degradation products appear in the faeces mainly as stercobilinogen and its oxidation product, stercobilin. Under normal conditions small amounts of stercobilinogen are reabsorbed from the intestine and appear in the urine as a very similar substance, urobilinogen. When the liver is damaged the amount of urobilinogen present in the urine may be increased without a corresponding increase in the amount of stercobilinogen and stercobilin in the faeces. Various methods have been used to estimate the amounts of these substances present in the urine and faeces. The most satisfactory for clinical purposes is Watson's (1936 *a*) modification of Terwen's method in which urobilin and stercobilin are reduced to urobilinogen with ferrous hydroxide and are then estimated as urobilinogen by a colorimetric application of the Ehrlich reaction.

There are many unsettled problems in connexion with the source of these pigments, but their estimation in disorders of the blood has afforded useful information which could not have been gained by other means. The pigments are always increased in amount, particularly in the faeces, in conditions which are undoubtedly haemolytic, whereas up to the present, though there are many theoretical possibilities, no source of a significant increase in the amount of the pigments excreted has been proven, other than an increased breakdown of haemoglobin. In particular there is no evidence that they are formed (and on theoretical grounds it is unlikely that they can be) from hypothetical precursors of haemoglobin which have not entered red cells. At the present time, therefore, the available evidence points to the fact that an increased total excretion of urobilinogen indicates an increased rate of haemolysis, provided always that proper precautions have been observed in the collection of the stools and urine, and that they have been collected over a sufficiently long period. Estimations of the average daily excretion are quite unreliable if the patient does not have regular daily formed stools over a sufficient period. The normal amount of urobilinogen excreted daily has been given by Watson (1937) as from 100 to 250 mg. in the faeces and from 0.5 to 2.0 mg. in the urine. In this laboratory somewhat lower figures have been obtained for the faeces. In 26 patients with normal haemoglobin figures and no evidence of any blood disorder the average daily urobilinogen excretion in the faeces was 79 mg., the highest figure obtained being 150 mg. We cannot account for this discrepancy, but as the estimations were made on our series of patients without blood disorders and on our series of patients with refractory anaemia under strictly comparable conditions, we have accepted 150 mg. a day as the upper limit of normal.

Determination of the average daily urobilinogen excretion in 3-day periods were made on one or more occasions in 30 of the patients included in this report. The findings in some of them have already been reported by Barker (1938). The amounts of urobilinogen excreted in the faeces was within the

normal range in 15 patients, above our limit of normal (150 mg.) in 15, and above Watson's limit of normal (250 mg.) in 11. The figures in these abnormal cases varied from 172 to 570 mg., with an average value of 308 mg. in the 15 patients. Biopsies of the bone-marrow had been performed in 29 of the 30 patients; of eight patients with hypoplastic bone-marrow the figures were normal in three and above normal in five; of 17 patients with marrow of the partly mature cellular type the figures were normal in 10 and above normal in seven; of three patients with bone-marrow of the immature cellular type the figures were normal in two and above normal in one; and the figures were above normal in one patient with marrow of the myelosclerotic type. There does not, therefore, appear to be any correlation between the type of marrow and the level of urobilinogen excretion in the faeces.

If increased urobilinogen excretion in the faeces indicates an increased rate of destruction of haemoglobin, haemolysis above the normal rate was present in half of the patients in whom this test was done. This finding is quite in keeping with the incidence of haemosiderosis of the organs found *post mortem*. There are one or two other points of interest in this connexion. Some of these patients had slight or moderate fever at the time the urobilinogen excretion was estimated. In our patients, however, there did not appear to be any correlation between the amount of urobilinogen excreted and the presence or absence of fever. Further, in a small series of patients with pneumonia Erf and MacLeod (1940) found no significant increase in urobilinogen excretion in patients not treated with sulphapyridine. As far as the meagre evidence goes, there is no reason to suppose that the presence of fever affected the findings in our patients. In several patients considerable increases in the amount of urobilinogen excreted were seen in the few days following a transfusion. Patients in whom the figures were abnormal only after transfusions were not included in calculating the number of cases with abnormal excretion given above.

In the 15 patients in whom the figures were within normal limits, it is not possible to conclude that there was not an increase in the rate of haemolysis. In experiments on animals made anaemic by bleeding and in a few patients with anaemia in whom there was no reason to suspect an increased rate of haemolysis (Watson, 1938) the figures for urobilinogen excretion in the faeces appeared to be roughly proportional to the total amount of haemoglobin in circulation. Thus to exclude an increase in haemolysis in patients with anaemia it would be necessary to know the average urobilinogen excretion in patients with different levels of haemoglobin in the absence of an increase in the rate of haemolysis. Since all our patients were anaemic, and many were severely anaemic, it is likely, though not proven, that a greater proportion than the 50 per cent. mentioned above had some increase in the rate of haemolysis.

Lastly, though unfortunately determination of the icterus index or plasma bilirubin were usually not made at the same time as determinations of the urobilinogen excretion, in a few instances the urobilinogen excretion was



distinctly increased where the icterus index or plasma bilirubin remained normal throughout the time the patients were under observation. This observation suggests that an increased rate of haemolysis may be present without an increase in the icterus index or plasma bilirubin.

### *Haemolysin in Refractory Anaemia*

Methods for the qualitative estimation *in vitro* of substances lytic to red cells and of substances which inhibit lysis have been developed particularly by Ponder (1934), and the methods used in this study were the standard methods described by him.

*Haemolysin in urine.* The studies of Ponder (1921) and Abels (1934) have demonstrated the presence of a substance lytic to red cells in over 90 per cent. of specimens of human urine, and at some time in the urine of all normal subjects examined. Abels and Rhoads (1938) examined 92 specimens from eight patients with refractory anaemia and found no lysin in any specimen. This suggested that if lysin were excreted in the urine in refractory anaemia it must be in a bound or conjugated form. The urine was therefore hydrolysed at 100° C. at pH 1 for one hour and thereafter the presence of lysin was demonstrated in 94 per cent. of 38 specimens from eight patients with refractory anaemia. Seven of the eight are cases included in this report. These experiments were carefully controlled as regards pH and other factors, and the results were constant. It was further shown that the lysin in normal lytic urine was not destroyed by the method of hydrolysis, that normal non-lytic urine did not contain lysin after the same procedure, and that patients with pernicious and iron-deficiency anaemia excreted urine in which lysin was present without hydrolysis in almost the same proportion of urines as in normal persons. The meaning of this observation remains at the moment obscure.

*Haemolysin in plasma.* Continuing these studies, Abels has made some preliminary and unpublished observations on the extraction of lytic substances from plasma of normal persons and of patients with refractory anaemia. For this purpose from 10 to 25 c.c. of plasma were deproteinized with 96 per cent. alcohol. The filtrate was heated at 60° C. on a water bath to remove the alcohol and then hydrolysed for 10 minutes at pH 1. During hydrolysis some brownish flaky material separated and was filtered off. This precipitate contained substances which were inhibitory to haemolysis *in vitro*. From the filtrate two fractions were prepared by ether extraction, one containing all the organic ether-soluble acid substances and the other the organic ether-soluble neutral and phenolic substances. Ether was evaporated from the fractions in a water bath and the remaining precipitates were dissolved in saline as far as possible. After adjustment of the pH the haemolytic activity of these saline extracts was determined in standard red cell systems *in vitro*.

In eight normal persons the acid-containing fraction was feebly lytic in every case, but the neutral and phenolic fraction produced no haemolysis.

In eight patients with refractory anaemia both the acid fractions and the neutral and phenolic fractions were feebly lytic in every case. Thus the finding of a lytic substance in the phenolic and neutral fraction was a constant abnormality in all cases tested. This observation must be regarded as a preliminary one, needing further investigation and confirmation. The observations on normal and pathological plasma were made under strictly comparable conditions, and appear to indicate an abnormality in the lytic substances of the plasma of patients with refractory anaemia.

#### *Resistance of Red Cells of Patients with Refractory Anaemia to Saponin Lysis*

The methods were those of Ponder (1934), and for an explanation of the notation used to express the results reference should be made to this author's book. An R value of 1.0 indicates a normal resistance of the red cells to saponin lysis. An R value of less than 1.0 indicates that the red cells are more vulnerable than normal to lysis by saponin, and an R value greater than 1.0 indicates that they are more resistant than normal to lysis by saponin.

Ponder and Rhoads (1938) have shown that the red cells from cases of pernicious anaemia in relapse are more vulnerable than normal to lysis by saponin, sodium taurocholate, and sodium glycocholate (R value 0.61 to 0.75). Abels determined the R value in four cases of refractory anaemia and found that the red cells in this condition were slightly more resistant than normal to lysis to saponin (R values 1.08 to 1.15).

#### *Protective Power of Plasma against Saponin Lysis in Refractory Anaemia*

If a constant proportion of normal plasma is introduced into standard red cell lysis systems the time required for lysis to occur is increased. Thus normal plasma affords some protection to red cells against lysis by saponin. The protective power of the plasma was determined by one of us (R. R. B.) in four cases of refractory anaemia, and compared with that of plasma from a control normal person. In three of the four cases the protective power of the plasma was very slightly greater than that of a normal control. This appeared to be correlated with the fact that in the first three cases the output of urobilinogen in the stools was low; in the fourth case there was evidence of an increased rate of haemolysis, the faecal urobilinogen and reticulocyte count being constantly above normal, and the protective power of the plasma less than that of a normal control.

#### *Disturbances in Excretion of Glucuronates and Sulphates in Refractory Anaemia*

Aromatic hydrocarbons are rendered harmless in the animal body by a variety of mechanisms, for which the general term detoxication has been used. The best known examples of this process are the conjugation of the

substance in question with glucuronic acid to form glucuronates or with sulphuric acid to form ethereal sulphates (Young, 1939).

An attempt was made to discover whether these substances are excreted in normal amounts in patients with refractory anaemia, and the effect of a test dose of pyramidon on the total excretion of reducing substances was also examined. In five normal persons there was a distinct increase in the amount of total reducing substance excreted on the day on which pyramidon was given, followed by a decrease to the previous level on the following day. In seven patients with refractory anaemia a similar result was obtained in two, in three there was no increase during the two days after pyramidon was given, and in one there was a considerable increase on two occasions, but this did not appear until the day after that on which pyramidon was given. In the remaining patient, the greatest increase seen in any of these tests occurred progressively over the next three days. In some instances similar determinations were made after fermentation, and also after hydrolysis of the urine. The curves obtained were closely parallel to those for total reducing substances. These findings suggest that there is a disturbance in the excretion of non-fermentable reducing substances, including glucuronates, in the urine of patients with refractory anaemia. In a series of patients with pernicious anaemia there was an almost normal result in every case, and in a series of patients with leukaemia there was no increase after pyramidon in any case.

The total sulphates and the partition between inorganic, ethereal, and neutral sulphates excreted in five normal subjects and in three patients with refractory anaemia were also determined. The results in the five normal persons were reasonably constant. Of three patients with refractory anaemia, the figures in one agreed closely with those of the normals; in the remaining two the total amount of sulphates excreted was reduced and the partition ratio was disturbed.

These findings, so far as they go, suggest the existence of a disturbance in the excretion of sulphates and glucuronates in patients with refractory anaemia.

#### *Discussion*

It has been said that no man can observe without a hypothesis. Though any hypothesis of the aetiology of refractory anaemia must be extremely tentative, an attempt to summarize some of the facts and to formulate a hypothesis to connect them may be of value. The main observations which must be considered appear to be as follows.

1. Severe refractory anaemia may follow exposure to toxic substances, almost all aromatic hydrocarbons, but an apparently identical disorder may occur where there is no evidence of such exposure. This suggests that the disorder in these latter cases might result from the presence of excessive amounts of an endogenous toxic aromatic hydrocarbon, or from failure of the mechanisms which protect the haemopoietic system from endogenous aromatic hydrocarbons normally present in the body. That the body does contain

potentially haemotoxic aromatic hydrocarbons is shown by the production of anaemia in experiments on animals with the use of indol (Rhoads and Barker, 1938) and oestrin (Rhoads, 1939).

2. Of all persons exposed to aromatic hydrocarbons only a few develop a refractory anaemia. It is therefore necessary to postulate a conditioning factor or state, which is responsible for the susceptibility of certain persons to these haemotoxic substances. There is no evidence that this susceptibility is due to a hereditary or congenital predisposition. A tendency in contemporary writings to attribute susceptibility in almost every disease to dietary deficiency or endocrine disorder makes it wise to be critical of such suggestions in the case of refractory anaemia. Rhoads, however, in carefully controlled observations has shown that susceptibility of the haemopoietic system to indol (Rhoads and Barker, 1938) and to amidopyrine (Miller and Rhoads, 1937) may be conditioned by the use of a deficient diet. It is therefore possible that deficiency of one or more dietary factors is important in refractory anaemia, and, as in the case of anaemia due to deficiency of the liver factor, the deficiency might theoretically be an overt one, due to an obviously deficient diet, or a conditioned one due to an increased demand for some factor present in limited amounts in a normal diet. The diet had been obviously deficient in only a minority of our patients. The slight suggestion in our patients of an association between eunuchoidism, sexual cycles, and some cases of refractory anaemia could be interpreted as suggesting that sex hormones might act, under certain conditions, as haemotoxic substances.

3. Susceptibility to haemotoxic substances can presumably be attributed more specifically to a failure of the biochemical mechanisms whereby the normal person is protected from haemotoxins. The present state of knowledge as regards the detoxication of aromatic hydrocarbons has recently been summarized by Young (1939). Aromatic hydrocarbons are rendered inactive in the body by a variety of mechanisms, the best known being by conjugation with phenols and glucuronic acid to form glucuronates, or with sulphuric acid to form ethereal sulphates, by conjugation with cystine to form mercaptans, by acetylation, and by the breaking down of the whole molecule. In the case of benzol intoxication analysis of the urine of experimental animals indicates, according to Stekol (1935), the formation of ethereal sulphates and glucuronates. Oestrone and oestriol in human beings are also excreted as glucuronates (Cohen and Marrian, 1936). It is theoretically possible, therefore, that a breakdown of one or more mechanisms of detoxication would render a person susceptible to both endogenous and exogenous aromatic hydrocarbons. We have quoted some evidence for the existence of a disorder in the conjugation of amidopyrine in patients with refractory anaemia.

4. It is supposed that biochemical processes of detoxication, such as those quoted above, take place mainly in the liver. There is some morphological evidence of liver damage and biochemical evidence of impaired hepatic function in a proportion of cases of refractory anaemia. Since at present it

is not possible to exclude hepatic dysfunction by morphological or biochemical means, it is possible that this form of dysfunction is present in a greater proportion of cases than is indicated by the results of liver function tests.

5. In mild cases of refractory anaemia the blood picture closely resembles that seen in such liver diseases as cirrhosis. It is possible, therefore, that the first stage in the development of refractory anaemia is an associated liver dysfunction and the failure of some biochemical mechanism of detoxication. If the process continues, the anaemia becomes more severe and loses its resemblance to that of liver disease. If, however, a remission then sets in, in the majority of cases the blood picture returns to one resembling closely that of liver disease, and in certain cases this picture has persisted as long as the patients have remained under observation. Circumstantial as well as direct evidence appears to incriminate liver dysfunction as a factor of importance in refractory anaemia; and this suggests that further investigation, necessarily mainly biochemical, of liver function and the mechanisms of detoxication are needed in this disorder.

6. There is evidence of a considerably increased rate of haemolysis in a proportion of cases of refractory anaemia, and as discussed in a previous section, it is impossible to exclude a slight increase in the rate of haemolysis in the remainder. Haemolysis or the injection of haemoglobin, in otherwise normal organisms, leads to a compensatory hyperplasia of the bone-marrow. Hyperplasia was present in a considerable proportion of our patients at the time of biopsy, but in comparatively few at the time of autopsy. Two possibilities seem worthy of consideration in this connexion. Firstly, it is possible that when the breakdown of processes of detoxication postulated above occurs, haemotoxins produce a haemolytic anaemia, but for some reason, as for instance an abnormality in the breakdown products of haemoglobin, the compensatory hyperplasia of the bone-marrow fails or is insufficient. Secondly, it is possible that haemotoxins destroy not only circulating red cells, but also developing cells in the marrow, thus producing hypoplasia or one of the other changes seen in the bone-marrow at autopsy.

A working hypothesis of the aetiology of refractory anaemia is, therefore, that the disorder is due to a conditioned susceptibility to toxic substances, usually exogenous or endogenous aromatic hydrocarbons, associated with hepatic dysfunction, a failure of biochemical mechanisms of detoxication, and the circulation of haemolytic substances, and that these haemolysins cause either an abnormal form of haemolysis and thus an abnormal reaction in the marrow, or that they destroy both circulating red cells and developing cells in the marrow, thus producing hypoplasia or other abnormal forms of marrow.

#### *The Treatment of Refractory Anaemia*

While the aetiology of refractory anaemia remains unknown, treatment must necessarily be empirical and unsatisfactory. In the meantime certain experimental observations have given a lead as to the type of therapy which

may reasonably be used in these disorders. Hypoplasia of the bone-marrow has been produced in experimental animals by two chief methods—by the injection of toxic substances, benzol (Selling, 1911), bacterial toxins (Cunningham and Doan, 1922), and pyramidon (Climenko, 1936), and by the use of deficient diets (Doan, 1922; Emmel and Streicher, 1930; Jordan, 1936; György, Goldblatt, Miller, and Fulton, 1937). Also Rhoads has shown that susceptibility of the haemopoietic system to indol and amidopyrine can be conditioned by means of a diet deficient in the vitamin B complex. There is, therefore, a rational basis for removing patients with refractory anaemia from exposure to any even potentially toxic substances, for providing them with a diet adequate in all respects, and for supplementing the diet with accessory food substances, particularly the vitamin B complex. Apart from these measures, blood transfusion must be the mainstay of treatment.

*Removal from exposure to toxic substances.* In this series of patients no single measure appears to have had more effect in improving the prognosis in patients with refractory anaemia than their removal from exposure to toxic substances. Direct and detailed inquiry should be made about patients' work, habits, home environment, and consumption of medicine; and if any exposure to a potentially toxic substance, particularly any substance containing a benzene ring, is discovered, this should immediately be terminated. Habitual consumers of sedatives and analgesics should be induced if possible to abandon these drugs completely. If drugs of this type are needed, they should be limited to bromides, codein, and if necessary, preparations of opium. We have already quoted several examples of patients whose condition deteriorated after single doses of pyramidon and similar drugs.

### Case Report

Severe purpura and anaemia. Terminally almost complete granulopenia, apparently produced by two doses of pyramidon.

*Case 40.* An American schoolboy, aged 11 years, had a crop of spontaneous bruises one week before admission. His previous history and family history appeared unimportant, and his diet had been good, except that he ate no citrus fruits. His gums began to bleed and he became pale and dyspnoeic on exertion. There was no history of any exposure to toxic chemicals.

*Physical examination.* Temperature 102·8° F., pulse 136. Well developed and well nourished, but very pale. Petechiae and very numerous ecchymoses, especially on the legs and around the lips; retinal haemorrhages; bleeding gums. Tongue normal. Systolic murmur at apex. Blood-pressure 120/65. No enlargement of spleen or lymphatic glands. No neurological abnormality.

*Laboratory investigations.* Blood count on admission, red cells 1,200,000 per c.mm., haemoglobin 25 per cent., colour index 1·0, mean corpuscular volume 95 cub.  $\mu$ , white cells 1,650 per c.mm.; differential count, polymorphonuclear leucocytes 24 per cent., lymphocytes 68 per cent., monocytes 2 per cent., eosinophils 6 per cent.; film, occasional macrocytes. Blood ascorbic acid 0·32 mg. per 100 c.c. Radiograph of chest, normal. Stool, many cysts and a few vegetative forms of *Giardia lamblia*. Wassermann reaction negative. Agglutination tests for *B. Melitensis* and Widal reaction negative.

*Further course.* The patient had irregular high fever up to 105° F. He remained very ill with fresh bleeding and purpura. Intensive treatment with intravenous ascorbic acid raised the blood-level to 1.7 mg. per 100 c.c., but had no significant effect on the clinical picture or platelet count. His white cell count at three- or four-day intervals was 1,650, 1,450, 2,750, and 1,600 per c.mm. On the day of this last count he was given 1.2 gm. of pyramidon for an attack of diarrhoea, and this treatment was repeated three days later. On the day after the second dose his white cell count was 750 per c.mm., with 4 per cent. of polymorphonuclears and 96 per cent. of lymphocytes. The patient died on the following day, three and a half weeks after his first symptom. His haemoglobin while he was under observation was never lower than 25 per cent.

Patients with refractory anaemia should also avoid exposure to strong sunlight, and should not be exposed to ultraviolet light or therapeutic X-irradiation. We have never seen ill-effects follow exposure to X-irradiation in amounts necessary for ordinary diagnostic procedures, including gastric fluoroscopy.

*Dietary measures and the use of dietary supplements.* Patients with refractory anaemia, except when acutely ill, have usually enough appetite to eat an adequate diet. This should include meat, fresh fruit, vegetables, and milk. In the course of this study a large variety of dietary supplements and other preparations has been tried. No definite effect has been observed with any of them, with the possible exception to be mentioned below, of large doses of baker's yeast. As already recorded, there was a history of definite improvement following therapy with liver extract in several patients early in the course of their disease. It would therefore seem wise to treat every patient with refractory anaemia with at least one prolonged course of injections of liver extract in as unrefined a form as possible. Recently we have treated five patients with large doses (90 to 120 gm.) daily of bakers' yeast by mouth for periods of two to six months. The condition in three of these five patients has remained stationary, in one it has shown distinct improvement, while one is clinically well, having had no transfusions for a year. Furthermore, in one patient reticulocyte counts suggested the existence of some activity in bakers' yeast not present in brewer's yeast. Coincidence cannot be excluded, but there is a suggestion that the disorder in these five patients has taken a more benign course than would have been expected in five unselected cases. In the absence of any specific therapy we believe that the prolonged use of large doses of bakers' yeast (90 to 120 gm. or more by mouth daily) is worthy of trial in these disorders.

*The use of blood transfusions.* Several questions arise in connexion with the use of blood transfusions in refractory anaemia. This disease is not uniformly a hopeless one; some improvement or a remission occurs occasionally, even in cases with severely hypoplastic marrow, after transfusions have been given over a considerable period. It is therefore justifiable to begin transfusions in every case, when they appear necessary, and to continue them however bad the outlook may seem.

Throughout this investigation transfusions have been given sparingly. They have been given frequently enough to keep their recipients free from obvious symptoms of anaemia, but no attempt has been made to raise the blood count to normal levels. Though there is some variation in individuals, patients who have had the disease for some time are usually comfortable in bed with a haemoglobin level at or above 30 per cent.; patients up and about usually require to be kept at slightly higher levels. A number of patients in this series had been given repeated transfusions to raise their haemoglobin to levels near normal, before they came under our observation. We have never seen any permanent benefit follow this procedure, and believe that in severe cases the attempt may actually be dangerous. Scott (1939 *b*) has recently recommended massive transfusions to raise the haemoglobin to a level of 70 or 80 per cent. in what he refers to as 'chronic panmyelophthisis with macrocytic anaemia'. This would correspond in our nomenclature to cases with a partly mature cellular marrow which are pursuing a relatively benign course. This author states that such patients may retain for considerable periods the level of haemoglobin reached after massive transfusions.

In text-books and other publications the statement is repeatedly made that transfusions stimulate the bone-marrow. There is, as far as we know, absolutely no evidence for this assertion. What little evidence there is suggests indeed that transfusions may depress the function of the marrow. It has been claimed, for instance, that the production by means of transfusions of artificial plethora in normal animals depresses erythropoiesis, as indicated by the level of the reticulocyte count, and may even produce hypoplasia of the marrow (Robertson, 1917).

The Carnot phenomenon (Carnot and Delfandre, 1906), of which the existence has been disputed (Müller, 1912; Gordon and Dubin, 1934), suggests that there may be a factor present in the plasma after haemorrhage which stimulates erythropoiesis. For this reason two patients have been given repeated transfusions from a single donor. One was a patient, under the care of Dr. Donald Hunter, with a moderately severe refractory anaemia with hypoplastic marrow, observed by one of us (R. R. B.) in London. This patient was given three transfusions, each of 300 c.c., from a donor who had previously been made slightly anaemic by the removal of 600 c.c. of blood. Improvement in this patient's condition began about this time and has continued. At the present time, two years later, this patient's blood count is within normal limits in every respect. A second patient, with a very severe refractory anaemia, seen in New York, but not included in this series, was given six pints of blood taken from one donor over a period of 17 days, with no apparent harm to the donor or benefit to the recipient.

That transfusions may have some action, apart from simply replacing blood, is indicated by the variable effect which transfusions from different donors may have in a single patient. Little is known about this subject, and it is an aspect of the therapy of refractory anaemia which merits further



investigation. In certain patients, usually those with a severe disorder, transfusions are followed by a rise in temperature, an increased excretion of urobilinogen, and a rapid disappearance of the increased haemoglobin level attained after transfusion. This suggests that in these patients an increased rate of haemolysis follows the transfusion. In other patients, usually those with a milder disorder, a previously raised temperature may fall to normal after a transfusion, and the subsequent increase in haemoglobin then usually persists for a considerable time. This suggests that in these patients the rate of haemolysis is slowed after a transfusion. We believe, therefore, that in all but the mildest cases transfusions should be given as sparingly as possible.

Patients with severe refractory anaemia appear especially susceptible to post-transfusion reactions. In the treatment of the cases in this series, not more than 500 c.c. of blood has usually been given at a time, and whole unaltered blood from a donor of identical group has been given rapidly by a multiple syringe method. We believe this to be the ideal method in patients with a severe refractory anaemia, and the one which reduces post-transfusion reactions to a minimum.

*The treatment of complications in refractory anaemia.* The complications of this disorder are often distressing and difficult to treat. The use of measures other than local treatment to control bleeding have appeared uniformly unsatisfactory, except that transfusions sometimes appear to diminish it. We have seen no good effect from the intravenous injection of so-called haemostatics in this condition. Bleeding from the gums in some cases can be controlled by applications of silver nitrate; in others the bleeding appears quite uncontrollable. Patients with a tendency to bleed from the gums should use mouth-washes, but not a tooth-brush. Bleeding from the nose can often be controlled by the application of a pledget of dry cotton-wool direct to the bleeding-point and the application of light counterpressure. Wholesale packing of the nasal cavity is often less effective, and further bleeding usually occurs when the packing is removed.

Infected and necrotic areas should be treated as conservatively as possible by the methods usually employed in the treatment of infections. The affected areas rarely contain pus, and operative measures should be avoided or delayed as long as possible. If operative measures are undertaken, haemostasis should be secured with the utmost care, and a donor of compatible type should be available.

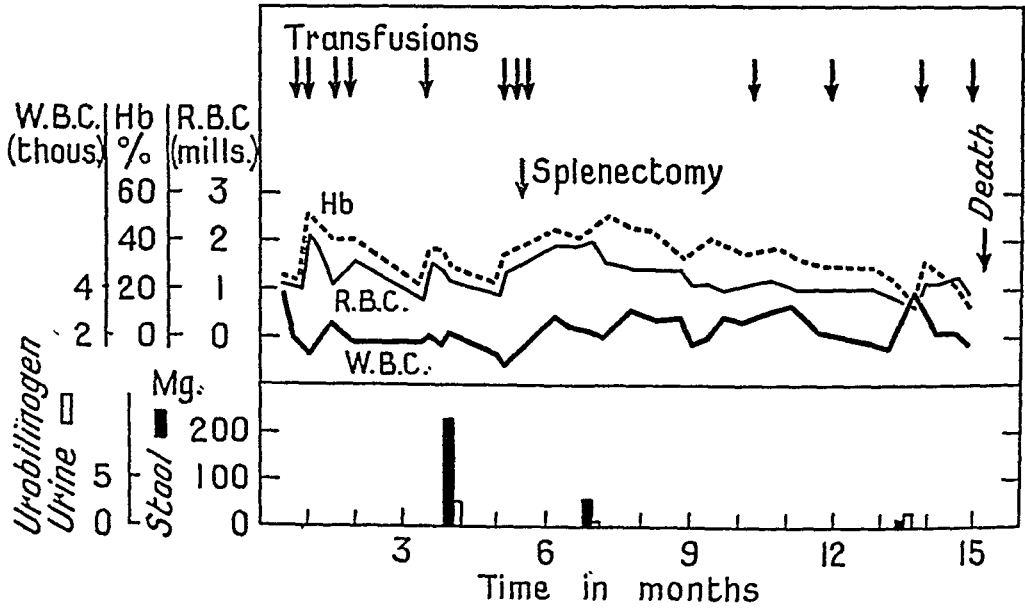
*The place of splenectomy in the treatment of refractory anaemia.* Splenectomy may be considered in a very small group of patients with refractory anaemia, and then only as a last resort. There are occasional patients with marrow of the partly mature cellular type in whose disorder haemolysis, as indicated by an increased excretion of urobilinogen, a high reticulocyte count, and the quick disappearance of the effect of transfusion, appears to be particularly rapid. In such patients the rate of haemolysis may be diminished by splenectomy, and the effect of transfusions may thus be prolonged (see Fig.).

There is no evidence that splenectomy has any other effect on the course of the disease, and it should not be considered in the absence of a partly mature cellular marrow and an increased excretion of urobilinogen.

Case Report

Refractory anaemia with hypocellular marrow and evidence of an unusual rate of haemolysis. Reduction in rate of haemolysis after splenectomy.

Case 41. A German-French housewife who had lived in America for 52 years first complained of dyspnoea and occasional pain 18 months before



Case 41. The figure shows the effect of splenectomy on the course of a case of refractory anaemia. The operation was followed by diminution in the rate of excretion of urobilinogen and diminished need for transfusions. There is no evidence that splenectomy has any other effect in this condition, and it should be considered only in cases in which there are special indications for its performance.

admission to the Rockefeller Institute Hospital. Her family history appeared unimportant. She had had several fevers in childhood, including typhoid fever. She stated that she had bruised easily since the menopause at the age of 42 years. Her diet had been an adequate one. Ten months before admission she had noticed throbbing in the head and pounding in the ears. About this time her hair was dyed for the first time with a paraphenylenediamine hair dye. One week later she had nose-bleeding. Two months later she had a further hair-dye treatment and a permanent wave, and shortly after this complained of dyspnoea and palpitation, and was noticed to be pale. Three months before admission to the Rockefeller Hospital she was admitted to the Mary Immaculate Hospital in Jamaica, where she was found to have severe anaemia with red cells 1,100,000 per c.mm., haemoglobin 25 per cent., colour index 1.2, white cells 4,100 per c.mm. The reticulocytes varied from 0.5 to 8.0 per cent. A bone-marrow biopsy was performed and the patient was treated with iron, liver extract, yeast, and given four blood transfusions with only temporary benefit. The patient became paler and weaker, and returned to New York.

*Physical examination.* Temperature 99.4° F., pulse 100. Moderately obese woman. Pale with yellowish skin and conjunctivae. Some large subcutaneous ecchymoses and a few petechiae, and a few small retinal haemorrhages. Tongue normal. Haemic murmur. No enlargement of liver or lymphatic glands; tip of spleen just palpable. Oedema of legs. No neurological abnormality.

*Laboratory investigations.* Blood count on admission, red cells 800,000 per c.mm., haemoglobin 20 per cent., colour index 1.19, mean corpuscular volume 125 cub.  $\mu$ .; reticulocytes 3.1 per cent., platelets 100,000 per c.mm., white cells 1,900 per c.mm.; differential count, polymorphonuclear leucocytes 39 per cent., lymphocytes 54 per cent., monocytes 3 per cent., eosinophils 2 per cent., basophils 1 per cent. Wassermann reaction negative. Icterus index 5. Haemolysis of red cells in saline began at 0.44 per cent. and was complete at 0.34 per cent. (control 0.44 to 0.34 per cent.). Test meal, free hydrochloric acid present. Average daily urobilinogen excretion, urine 2.7 mg., stools 230 mg. Radiographs of bones showed hypertrophic arthritis only; radiograph of chest normal.

*Biopsy of sternal marrow.* A section sent from the Mary Immaculate Hospital, Jamaica, showed a very fatty aplastic marrow, with small scattered islands of blood formation.

*Further course.* During her stay in hospital the patient had slight fever with occasional rises to 101 or 102° F. Up to 4 per cent. of myelocytes were seen occasionally in her blood films. A period of intensive parenteral liver therapy produced no improvement. In view of the persistently raised reticulocyte count (1.8 to 5.8 per cent.) and the increased urobilinogen excretion in the stools (230 mg. per diem), it was decided to remove the patient's spleen, and she was transferred to the New York Hospital. Under ethylene anaesthesia the spleen, weighing 257 gm., was removed by Dr. Andrus; the patient received a transfusion before and after the operation and her post-operative course was uneventful. The patient was readmitted to the Rockefeller Institute Hospital five weeks after the operation. She had been home and had been up and about. Her scar was well healed. She was pale, and a haemic murmur and a few old retinal haemorrhages were the only physical signs found. Her blood count five weeks after the last transfusion was, red cells 1,900,000 per c.mm., haemoglobin 40 per cent., colour index 1.05, white cells 2,400 per c.mm., the last being higher than at any time since she was first admitted. Her average daily urobilinogen excretion was 60 mg. in the stools and 0.2 mg. in the urine. There was, therefore, evidence that the rate of haemolysis had been decreased, but the reticulocyte count continued to be above normal, varying from 2.9 to 6.6 per cent. The patient was discharged to her home and given liver extract by mouth. Her general condition remained good for four months after her operation. At the end of this time her blood count slowly declined and her symptoms returned. Eight months after the splenectomy she was readmitted. She had had some nose-bleeding and her blood count was, red cells 900,000 per c.mm., haemoglobin 26 per cent., platelets 54,000 per c.mm., white cells 1,400 per c.mm. The average daily urobilinogen excretion was 20 mg. in the stools and 1.1 mg. in the urine. She was treated with liver extract and ascorbic acid, given several blood transfusions, and discharged unimproved after seven weeks. She died one week later, but no autopsy was performed.

For effects of splenectomy see also cases 4 and 39.

*Summary*

The records of 66 cases of refractory anaemia were examined in an attempt to discover the factors which appeared to be of importance in the aetiology of this condition.

1. Race, sex, age, and family history appeared to have no influence on the occurrence of the disease.

2. A possible association with eunuchoidism, menstruation, and the menopause was noticed in a few instances.

3. The incidence of achlorhydria was below the normal. Slight atrophy of the mucous membrane of the edges only of the tongue was seen in a few instances. No disorders of the nails or nervous system were seen in any of these patients.

4. Half of the patients were known to have been exposed to potentially haemotoxic substances in the form of aromatic hydrocarbons, and one to X-irradiation and radium.

5. There was a suggestion that exposure to sunlight, ultraviolet light, and X-irradiation made worse the condition of a few patients with refractory anaemia.

6. Slight or moderate degrees of macrocytosis were observed in examples of all four types of refractory anaemia, and an increasing degree of macrocytosis usually indicated the onset of a spontaneous remission.

7. Pigmentation of the skin and, occasionally, haemochromatosis occurred as complications of refractory anaemia of the type with partly mature cellular marrow.

8. The results of liver function tests suggest that impairment of liver function may be an important factor in the aetiology of refractory anaemia.

9. The simultaneous excretion by patients with refractory anaemia of coproporphyrins I and III indicated that the disturbance of pigment metabolism in this condition is similar to that found in diseases of the liver and in certain forms of exogenous poisoning, and unlike that found in pernicious anaemia and haemolytic jaundice.

10. The rate of excretion of urobilinogen in the faeces was increased above the normal levels in some cases of all four types of refractory anaemia and in half of all the patients examined.

11. The haemolytic substances normally present in human urine are present in a bound or conjugated form in the urine of patients with refractory anaemia.

12. The red blood-cells in refractory anaemia were found to be slightly more resistant than normal to lysis by saponin.

13. Preliminary observations of a disturbance in the excretion of sulphates and in the excretion of glucuronates after a test dose of pyramidon suggest the existence of an abnormality in the processes of detoxication in patients with refractory anaemia.

14. The significance of these observations is discussed and a tentative hypothesis formulated.

15. The single measure which appeared of greatest importance in the treatment of refractory anaemia was to prevent the exposure of patients with this condition to any potentially haemotoxic substances or influences, including all drugs of the aromatic hydrocarbon series. The usefulness of blood transfusions, dietary supplements, and splenectomy is discussed.

We wish to express our gratitude to the patients, whose co-operation during the course of a trying and usually fatal illness made this study possible; to all those who in their several capacities have assisted in the care of the patients and in the work of the laboratory; and to the members of the staff of the Rockefeller Institute and Hospital, whose criticism and advice has been both helpful and stimulating. In particular we are indebted to Dr. Konrad Dobriner for the study of the excretion of porphyrins, and to Dr. J. C. Abels for the study of haemolysins in the urine and plasma. Professor H. M. Turnbull has kindly read the section on the histology of the bone-marrow and made criticisms and suggestions. One of us (R. R. B.) gratefully acknowledges his debt to the Rockefeller Foundation for an opportunity of working in America, and to the Director and members of the staff of the Hospital of the Rockefeller Institute for Medical Research for their hospitality and assistance.

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buttocks and pressure points were spared, and the rash did not itch nor was it painful. There were no petechial haemorrhages. There was a patch of early herpes febrilis beneath the nasal septum. His legs were flexed and immobile, Kernig's sign was present on both sides, and there was marked neck rigidity and some head retraction. The cranial nerves were normal except for changes in the optic disks. Visual acuity was normal in both eyes, there was no photophobia, and both fields were full to confrontation. The right disk was uniformly pink and the edges could not be seen at all. The vessels rose from an indefinite blur, and the appearance was that of a papillitis. There was no measurable swelling, but the pit was obscured. On the left the temporal edge was well defined, but the nasal half showed the same changes as in the right eye. The retinae and vessels were normal.

Passive movement of the limbs, especially the legs, caused intense pain, and the patient cried out when the thighs or legs were stretched. Pain prevented active movement. All the upper arm muscles were very tender, especially the pectorals, deltoids, and latissimi dorsi; the quadriceps femoris, adductors, and the glutei were similarly affected. The tenderness was less marked peripherally. The muscles felt firm, and there was some abdominal guarding, the latter normal within the limits of pain. No fibrillation or fasciculation was seen. The tendon reflexes could all be obtained, but were very sluggish. The superficial reflexes were brisk and the plantar responses flexor. There was no ankle clonus.

There was severe hyperalgesia of the trunk. Gentle elicitation of the abdominal reflexes caused a cry, and running the finger-nails lightly over the abdomen was almost unbearable. This was not so marked peripherally and the limbs were spared. There was hyperaesthesia on the trunk and over the area of the rash. Pin-prick was felt as unusually severe pain. Elsewhere light touch was normally appreciated, and joint sense and vibration were normal in the fingers and toes. There was tenderness over both great sciatic nerves, but the other nerves were not tender nor did they seem enlarged. The other systems were normal. The tongue was coated with white fur, the throat was a little injected, there was no adenitis, and the sites of inoculation were not painful. The urine was normal.

#### *Lumbar Puncture.*

Cerebrospinal fluid, clear and colourless.

Pressure, over 300 mm. of water.

Cell count, 400 per c.mm. (200 polymorphonuclears, 200 lymphocytes).

Protein, 100 mg. per 100 c.c.

Globulin, slightly increased.

Sodium chloride, 600 mg. per 100 c.c.

Culture, sterile.

The high-pressure reading was due in part at least to the abdominal rigidity which was present. A blood culture was sterile.

When he was seen on admission it was thought that the onset 36 hours after the injections was too rapid to inculcate the inoculations, and too long for an allergic reaction to foreign protein. The picture was that of an acute dermatoneuromyositis with a superimposed meningitis of unknown origin. The 50 per cent. count of polymorphonuclear leucocytes in the cerebrospinal fluid indicated an active meningeal infection. Papillitis is not described in acute dermatoneuromyositis, but with signs and symptoms of a neuritis elsewhere a similar inflammation of the optic nerves seemed possible. The



opinion was therefore expressed that the case was an unusual combination of acute dermatoneuromyositis and an acute meningitis, perhaps due to an infection introduced by the inoculations. As a diagnosis could not be made with certainty, treatment was withheld, and an injection of morphine sulphate gr.  $\frac{1}{4}$  was given to relieve the great pain and discomfort.

On 8.4.1940 the condition had improved, but there was a little pain in the muscles. The rash, which was now itching, had spread to the face, which was diffusely flushed, but elsewhere the erythema had faded considerably. The hyperaesthesia and hyperalgesia were less marked, and neck rigidity was still present, but greatly diminished. Kernig's sign was present. The right optic disk was rather more swollen and hyperaemic, but the left was unchanged. There was blurring of vision, and the visual acuity was 6/9 in both eyes. The fields were unchanged. The proximal limb and trunk muscles were still tender to palpation, and there was no other change in his clinical state.

#### *Lumbar Puncture.*

Pressure, 140 mm. of water.

Cell count, 3,800 per c.mm. (88 per cent. red cells, 6 per cent. (228) lymphocytes, 6 per cent. (228) polymorphonuclears).

Protein, 80 mg. per 100 c.c.

Globulin, not increased.

Sodium chloride, 700 mg. per 100 c.c.

Lange curve, 0000000.

Wassermann reaction, negative.

The blood Wassermann reaction was negative.

On 9.4.1940 all symptoms had improved, and although pruritus was still present the rash was fading rapidly. The neck rigidity had gone and Kernig's sign was negative. There was less blurring of vision, but no change in the optic disks. Paraesthesia had disappeared, sensation was normal, and no other changes could be found in the nervous system. Vomiting began in the morning and continued through the day. The vomitus was at first food, and later clear fluid.

10.4.1940. Although the other symptoms were improving he vomited intermittently and had frequent rather loose stools. He became dehydrated and small rectal salines were given. Examination of the central nervous system was negative except for the papillitis, which seemed less marked. Visual acuity had returned to 6/6 in both eyes. A throat swab was cultured, but no haemolytic streptococci or other pathogenic organisms were grown. Blood count, red cells 5,540,000 per c.mm., haemoglobin 100 per cent., colour index 0.9, white cells 13,000 per mm.; differential count, neutrophils 65.5 per cent., eosinophils 1.5 per cent., basophils 0.5 per cent., lymphocytes 24.0 per cent., transitionals 8.5 per cent. Vomiting ceased in the evening.

On 12.4.1940 he felt quite normal. Glucose by mouth and rectal salines were discontinued. No specific treatment had been given.

#### *Lumbar Puncture.*

Pressure, 140 mm. water.

Cell count, 260 per c.mm. (150 polymorphonuclears, 110 lymphocytes).

Protein, 40 mg. per 100 c.c.

Sodium chloride, 700 mg. per 100 c.c.

Lange curve, 000000000.

Culture, sterile.

14.4.1940. There was branny desquamation on the chest and abdomen. The optic disks were still blurred, but otherwise the patient was well.

29.4.1940. He had remained well.

*Lumbar Puncture.*

Pressure, 120 mm. water.

Cell count, 16 per c.mm. (all polymorphonuclears).

Protein, 40 mg. per 100 c.c.

Sodium chloride, 700 mg. per 100 c.c.

Culture, sterile.

Intradermal injections of water, tetanus toxoid, T.A.B., and a mixture of these were all negative.

*Case 2.* J.I.B., aged 26 years, joined the service on 16.4.1940 and was then perfectly well. His past health had been good, and he had had no hay-fever, asthma, or idiosyncrasy to any foreign substance. He had been vaccinated unsuccessfully in infancy, but had never had any inoculations. Six weeks before the present illness began he had had a little pain down the back of the right leg, which had not stopped him working, and had cleared up in a few days. There was nothing noteworthy in his family history.

Two days after enlistment he was inoculated with 1 c.c. of tetanus toxoid and 0.5 c.c. of T.A.B. vaccine. That night he felt ill, and on getting out of bed was so weak that he collapsed. He then had a rapid onset of general malaise, fever, bi-frontal headache, and shooting pain in the neck, back, and down both legs, especially the right. Stretching the legs or neck caused a sharp pain in the neck and back. There was extreme generalized tenderness of the calf and thigh muscles, the trunk—where the abdominal muscles were especially affected and were rigid—and also of the shoulder girdle, but the joints were normal. The right sciatic nerve was tender and there were feelings of pins and needles in the right foot. There was marked neck rigidity, and Kernig's sign was present on both sides, especially on the right. Lasègue's sign was also present on the right. He was drowsy, but could be easily roused by speaking. There was a faint erythematous rash on the trunk, without any purpuric spots. The cranial nerves were normal and the optic disks showed no changes. The motor system was normal except for limitation of movement and loss of power caused by the tenderness. The only sensory change was a feeling of pins and needles in the right foot, with an area of hyperalgesia over that foot and the outer side of the right leg. The heart and lungs were normal, and the throat slightly injected. The abdomen could not be examined properly because of the muscular tenderness. In the evening his temperature was 101.8° F., pulse 84, and respiration 28. Next day his condition remained unchanged.

On 20.4.1940 he was admitted to hospital with a diagnosis of cerebrospinal meningitis, in view of marked neck rigidity and bilateral Kernig's sign. When seen he was hot, flushed, and sweating, but alert and mentally normal. Head retraction and neck rigidity were marked. There was pain in the back and down the back of the right leg, made worse by flexion of both hip-joints to a right angle. Both buttocks, thighs, and the proximal muscles of both arms were tender, but the distal muscles were spared. The site of inoculation was a little red and sore, the rash had faded, the throat was clear, and there was no adenitis. The affected muscles felt unusually firm. There was no fibrillation or fasciculation. Power and coordination were within normal limits if allowance was made for the pain and tenderness. The tendon

reflexes were all brisk and equal, the superficial reflexes present, and the plantar responses flexor. Except for an area of hyperaesthesia on the outer side of the right foot and lower leg the central nervous system was normal. The tongue was covered with heavy white fur, but otherwise clinical examination was negative.

*Lumbar Puncture.*

Cerebrospinal fluid, clear.  
Pressure, 180 mm. water.  
Cell count, 170 per c.mm., mostly red cells.  
Protein, 40 mg. per 100 c.c.  
Sodium chloride, 720 mg. per 100 c.c.  
Lange curve, 00000000000.  
Wassermann reaction, negative.  
Direct smear, no organisms seen.  
Culture, sterile.

Lymphocytes and polymorphonuclear leucocytes were also present in the cell count, but further details were not given.

Blood cultures showed a growth of *Staphylococcus albus*, thought to be a contaminant. The throat-swab culture grew no abnormal organisms.

On 21.4.1940 his general condition was the same, and except that the rash had faded completely the signs were unchanged.

By 22.4.1940 his condition had improved and the neck rigidity was slightly less marked, but he still resented flexion of both knees and hips beyond a right angle. Tenderness was still present in the right sciatic nerve, and there was still spontaneous pain down the back of the right leg. Muscular tenderness had disappeared.

23.4.1940. *Lumbar Puncture.*

Cerebrospinal fluid, clear and colourless.  
Pressure, 140 mm. water.  
Cell count, 110 per c.mm. (10 lymphocytes, the rest red cells).  
Protein, 40 mg. per 100 c.c.  
Sodium chloride, 730 mg. per 100 c.c.  
Culture, sterile.

26.4.1940. Improvement had been maintained, and the only abnormal sign was slight neck rigidity and some stiffness and tenderness of the legs. Intradermal injections of sterile water, tetanus toxoid, T.A.B., and a mixture of these did not produce any effect. Subsequent progress was uneventful, and after sick leave the patient returned to full duty.

*Case 3.* G.J.L., aged 20 years, had had no serious illnesses. He had been vaccinated successfully in infancy. There was no family history of asthma or other allergic disorder.

On 17.4.1940, on enlistment, he had a 'cold in the head' with coryza and slight malaise. He was passed Grade 1 and began full duty. In the morning of the 24.4.1940 he was inoculated with 1 c.c. of tetanus toxoid and 0.5 c.c. of T.A.B. vaccine, and was vaccinated. In the evening he felt vaguely ill, and bi-frontal and occipital headache began. Later he felt nauseated and had aching pain in both buttocks, which felt tender on pressure. Next morning he felt hot and wretched, the frontal headache had persisted, and there was slight stiffness of the neck with pain and tenderness in all the

shoulder muscles and in the buttocks. His eyesight was blurred and he felt drowsy. The arm had not been unduly sore.

On 26.4.1940 all the symptoms became worse. Neck rigidity and bilateral slight Kernig's sign were found, and a tentative diagnosis of cerebrospinal meningitis was made. When seen in hospital in the evening he was feverish, restless, and uncomfortable, and said that all his muscles were sore and tender. There was no rash. He resented movement of his arms and legs, and was uncomfortable when he moved in bed. His arm was not painful at the site of inoculation. He had moderate headache of a throbbing type in both frontal regions and said that his neck felt stiff. The blurring of vision was better and he could see normally. There were no other complaints. He was mentally alert, no neck rigidity was present, and Kernig's sign was negative. On inquiry it was reported that there had been moderate neck rigidity, with a slight but definite Kernig's sign on both sides throughout the previous day. The muscles of the shoulder girdle, abdomen, and buttocks were tender to palpation. The other muscles of the trunk, arms, and legs were normal. Stimulation of the skin of the abdomen produced a very unpleasant sensation. He winced at a pin scratch and when lightly pricked. The peripheral nerves were not tender. The central nervous system was otherwise normal except that the ankle-jerks were very sluggish. The other systems were normal, and there was no adenitis.

#### *Lumbar Puncture.*

Cerebrospinal fluid, clear and colourless.

Pressure, 70 mm. of water.

Cells, none were seen.

Protein, 25 mg. per 100 c.c.

Sodium chloride, 680 mg. per 100 c.c.

Lange curve, 0000000000.

Wassermann reaction, negative.

Culture, sterile.

Blood Wassermann reaction, negative. Blood culture, sterile. The urine was normal.

On the 27.4.1940 tenderness was still present in the glutei and latissimi dorsi, but the abdominal hyperaesthesia was less marked. In the afternoon the temperature rose to 103.4° F., and next day he had a cough with a little purulent expectoration and sharp intermittent pain in the left chest on deep inspiration. The respiratory rate was 24 per minute, and the chest expansion normal, but there was slight dullness in the left mid-axillary line with a hard friction rub just external to the apex beat and a few fine crepitations lateral to this area.

On the 29.4.1940 there were signs of a small effusion at the left base. His general condition was good, the cough had ceased, and all the other symptoms had subsided. On the 2.5.1940, except for the chest signs, which were unchanged, there was no abnormality. X-ray of the chest showed a small left basal effusion. A blood count showed, white cells 5,000 per c.mm.; differential count, neutrophils 56 per cent., lymphocytes 30 per cent., transitionals 11 per cent., eosinophils 3 per cent., basophils 0 per cent.

By 6.5.1940 the effusion was subsiding, and the patient felt well, but was coughing a little. Examination of the central nervous system was negative. The reflexes were less brisk than previously. Examination of the sputum for tubercle bacilli was negative.

*Lumbar Puncture.*

Cerebrospinal fluid, clear and colourless.

Pressure, 70 mm. water.

Cells, none were seen.

Protein, 25 mg. per 100 c.c.

On 8.5.1940 the chest was practically clear and there were no other signs or symptoms. X-ray of the chest showed that the left-sided effusion had subsided. Intradermal injections of tetanus toxoid, T.A.B., and a mixture of both produced no reaction. Subsequent convalescence was uneventful.

*Case 4.* L.J.K., aged 21 years, joined the service, was passed fit, Grade 1, and on 11.4.1940 was inoculated with 1 c.c. of tetanus toxoid and 0.5 c.c. of T.A.B. vaccine, and was vaccinated. He had had no previous inoculation, nor had he been vaccinated before. He had little or no reaction from the inoculations, and on 27.4.1940 was given a further injection of 1 c.c. T.A.B. vaccine. Subsequently, the arm was not sore and there was no malaise. The vaccination pustule was small and perfectly satisfactory. During 29.4.1940 he noticed tingling of the fingers and toes, with numbness in the hands. He felt well, but tingling persisted. On 3.5.1940 he felt ill and a severe left frontal headache began. He had pain in the muscles of the lower part of the back and in the limbs. There was no stiffness of the neck. He had a temperature of 101° F., the tongue was coated, and Kernig's sign was doubtfully present on the left. There was no other abnormality. Later the headaches became more severe and neck rigidity was found. On 4.5.1940 the headache and pains in the back and limbs persisted. He felt feverish, uncomfortable, disliked light, and had pain on flexion of the neck. Marked photophobia was present, there was considerable neck rigidity, and Kernig's sign was positive on the left. The patient was given 2 gm. of sulphapyridine at noon as he was suspected of having cerebrospinal meningitis, and was transferred to hospital.

When seen he had all the symptoms already described. On examination there was severe photophobia. There was a confluent erythema on the face, neck, and shoulders which were rather puffy and swollen. This rash faded on pressure, was painless, and there were no haemorrhages. Tenderness was particularly marked in the proximal limb muscles, and he grimaced with pain when they were handled. This tenderness was confined to the muscles, and the joints were all normal. The abdomen was rigid, and its skin and muscles were very tender on light palpation; neck rigidity was considerable, and it was impossible to bend his head forward. Kernig's sign was positive on both sides. Mentally the patient was drowsy and he could not give a continuous coherent story without frequent prompting. He responded correctly to questions, and was orientated in place and time. When examined the cranial nerves were normal, except for severe photophobia. Muscular power was much diminished owing to the pain and tenderness of the trunk and proximal muscle groups of the limbs. The tendon-jerks were all very sluggish, the triceps-jerk being absent on both sides, and the ankle-jerks obtained only on reinforcement. Otherwise the motor system was normal. There was hyperalgesia to pin-prick over the legs and trunk, especially over the abdomen, where a light scratch would cause him to cry out. Other forms of sensation were normal. There was no adenitis, the joints were normal, and the throat clear. Except that the tongue was heavily furred, the other systems were normal.

*Lumbar Puncture.*

Pressure, 180 mm. of water.

Cell count, 5 per c.mm., mostly lymphocytes, with one or two degenerate polymorphonuclears.

Protein, 60 mg. per 100 c.c.

Globulin, no increase.

Sodium chloride, 700 mg. per 100 c.c.

Culture, sterile.

Blood culture, sterile. Urine, normal.

On 5.5.1940 the temperature rose to 106° F., but fell to 103° F. after tepid sponging. There was no evidence of any chest infection or of any abdominal abnormality. All the other symptoms and signs were unchanged.

On 6.5.1940 the temperature settled rapidly and the patient became more comfortable. The rash was less marked. Muscle tenderness was also less and was confined to the abdomen, where hyperaesthesia was still present. The reflexes became more sluggish, and the only deep reflexes which could be elicited were the supinator jerks. All the others were absent even on re-inforcement. The abdominal reflexes were brisk and the plantar responses flexor.

On 7.5.1940 he felt well and had no headache. The erythematous rash on the face, neck, and shoulders had almost gone, and fine desquamation had begun. Neck rigidity was still present, muscular tenderness had disappeared, and Kernig's sign was negative. All the tendon reflexes were absent and the plantar responses flexor. Sensation was normal, and all hyperaesthesia had disappeared.

*Lumbar Puncture.*

Pressure, 140 mm. of water.

Cells, 16 per c.mm. (11 red cells, 5 lymphocytes).

Protein, 40 mg. per 100 c.c.

Lange, 00000000000.

Wassermann reaction, negative.

Blood culture, sterile. Blood Wassermann reaction, negative.

Blood count, white cells 5,400 per c.mm.; differential count, neutrophils 60 per cent., lymphocytes 31 per cent., transitionals 7 per cent., eosinophils 1.5 per cent., basophils 0.5 per cent.

By 9.5.1940 there was great improvement. The reflexes were slowly returning but the triceps and ankle-jerks were still absent. Beyond a heavy desquamation on the head and neck no abnormality was found. Intradermal tests, as in the other cases, were negative. Convalescence was rapid, and after two weeks sick leave he returned to duty.

*Case 5.* C.L.L., aged 20 years, had had good health and was passed fit, Grade 1, on 24.6.1940. He had not been inoculated or vaccinated before. There was no family history of asthma, migraine, or any allergic manifestation.

On 24.6.1940 he was inoculated with 1 c.c. of tetanus toxoid and 0.5 c.c. of T.A.B. vaccine, and was vaccinated. There was no undue reaction, and on 1.7.1940 he was given a further 1 c.c. of T.A.B. vaccine at 4 p.m. At 6 p.m. he had a severe bi-frontal headache and went to bed early. He was awakened by a headache, and felt tired and a little sick. Next day he had the same bi-frontal headache, felt generally tired, and vomited once. His abdomen felt very sore and he had a pain in his abdominal muscles as if he had been kicked. The temperature was 102° F.

On 3.7.1940 the temperature was 102.6° F. There was slight neck rigidity, a doubtfully positive Kernig's sign, brisk tendon reflexes, and flexor plantar responses. He was transferred to hospital as a possible case of cerebrospinal

TABLE I

Case number	1	2	3	4	5
Date of onset	5.4.40	18.4.40	24.4.40	29.4.40	1.7.40
Age (in years)	23	26	20	21	20
Onset after T.A.B.	36 hr.	12 hr.	12 hr.	9 days or 48 hr.	7 days or 2 hr.
Maximum temperature (degrees F.)	100.0	101.8	104.0	106.0	102.6
Headache	0	+	+	+	+
Nausea or vomiting	+	+	+	0	+
<i>Meningeal Signs</i>					
Neck rigidity	++	++	+	+	+
Kernig's sign	++	++	+	+	+
<i>Peripheral Nerves</i>					
Paraesthesia	+	+	0	+	0
Hyperaesthesia	+	+	+	+	0
Nerve tenderness	+	+	0	0	0
<i>Muscles</i>					
Tenderness	++	+	+	+	+
Diminished reflexes	+	0	+	+	0
<i>Skin changes</i>					
Rash	++	+	0	+	0
Desquamation	+	0	0	+	0
<i>Eye Signs</i>					
Visual changes	+	0	+	+	0
Optic disk changes	++	0	±	0	0
Cerebral symptoms	0	0	+	+	0
Cerebrospinal fluid cells	{ 200 lymphs. 200 polys.	* 180 cells	† 0 cells	5 lymphs. and polys.	† 10 red cells
Cerebrospinal fluid culture	0	0	0	0	-
Blood culture	0	0	-	-	-
White blood cells per c.mm.	12,000	-	5,000	5,400	-

\* Mostly red cells; polymorphs and lymphocytes also present, but proportions not stated.

† Obtained when meningeal symptoms had subsided.

TABLE II

Case number	1	2	3	4	5
History of allergy	0	0	0	0	0
Previous vaccination	Infancy (unsuccessful)	Infancy	Infancy	0	0
Previous inoculations	0	0	0	0	0
Onset after vaccination	36 hr.	-	12 hr.	9 days	7 days
Onset after tetanus toxoid	36 hr.	12 hr.	12 hr.	9 days	7 days
Onset after 0.5 c.c. T.A.B.	36 hr.	12 hr.	12 hr.	9 days	7 days
Onset after 1 c.c. of T.A.B.	0	0	0	48 hr.	2 hr.

meningitis. On admission he still complained of a continuous bi-frontal headache made worse by movement, and of stiffness and pain in the neck. The muscles of the upper abdominal wall felt sore and were tender, but the other muscles were normal. He had no rash, no superficial hyperaesthesia, and no paraesthesia or numbness. There were no visual symptoms. On

examination he was normal mentally. There was no rash, and the vaccination vesicle was satisfactory. The only abnormalities found were tenderness of the abdominal wall, and moderate neck rigidity with pain on flexion. Kernig's sign, though not marked, was positive on both sides. The other systems were normal, and the urine was normal.

*Lumbar Puncture.*

Pressure, 120 mm. of water.  
Cells, 10 red cells per c.mm.  
Protein, 30 mg. per 100 c.c.  
Globulin, no increase.  
Sodium chloride, 740 mg. per 100 c.c.  
Lange curve, 0000000000.  
Wassermann reaction, negative.  
Culture, sterile.

On 5.7.1940 he was free from symptoms and signs and had no pyrexia. Subsequent progress was uneventful.

*Discussion*

*Aetiology.* This distressing but apparently benign syndrome began in each of the five patients within a few days of his being called up to the same Royal Air Force station. Furthermore, four of the cases occurred in the same month. The first was the most severe, and the last, which appeared eight weeks after the others, was milder. As every new recruit was inoculated on enlistment the injections might well be coincidental, and there is really no *prima facie* reason to inculcate them. Without them the outbreak would assume the characteristics of a small localized epidemic in a group of healthy men brought from all parts of the country into close proximity with each other. It would, nevertheless, be well to investigate any possible role played by the inoculations. Reference to Table II shows that vaccination as a cause is excluded by Case 2. Cases 4 and 5 did not have symptoms until after the second T.A.B. inoculation. Thus while the time of onset of symptoms after injection of tetanus toxoid ranged from 12 hours to 9 days, after T.A.B. inoculation it only varied within 48 hours. In the first four cases the onset appeared within 12 to 48 hours, with a mean of 27 hours. This is much more constant than the interval after injection of tetanus toxoid, so that it is more reasonable to blame the T.A.B. vaccine; but it will be noticed that although in Case 5 no symptoms were observed after the first T.A.B. injection, they occurred two hours after the second, and the same batch of vaccine was used for both injections. It is therefore unlikely that the causal agent was actually present in the T.A.B. vaccine. The fact that many thousands of recruits were inoculated under identical conditions, and that scores received T.A.B. vaccine from the same batches as the patients and yet did not show this syndrome, supports this conclusion. Either, therefore, the syndrome was unrelated in any way to the T.A.B. vaccine or it was precipitated by it in already predisposed subjects. A consideration of the time of onset of the symptoms shows that the second possibility is the more reasonable.



The time of onset of the illness after the inoculations, the presence of meningitis with a lymphocytic and polymorphonuclear pleocytosis in the cerebrospinal fluid in three cases, the absence of eosinophilia, and the negative results of intradermal injections, are all unusual in an allergic reaction. Furthermore, no serum was inoculated, so that sensitization to a foreign substance is unlikely to have been the cause of the syndrome. On the other hand, the whole character of the illness, and the epidemic nature of its occurrence, suggests an infective agent as the cause. No organisms were ever seen or cultured, so that the possibility of a virus disease must be considered. With this in mind, cerebrospinal fluid was collected for injection into monkeys, but owing to the conditions prevailing shortly after the outbreak of war no animals were available and the opportunity was missed. Proof of the nature of the illness is therefore lacking, but the following points in favour of a virus infection are significant.

The rapid onset with severe bi-frontal headache, malaise, and sweating is common to many acute illnesses, but the subsequent meningeal syndrome cannot be confused with simple meningism. In three cases there were both lymphocytes and polymorphonuclear leucocytes in the cerebrospinal fluid, while in the two cases where no pleocytosis was found, the meningeal signs had subsided before lumbar puncture was performed. Leucocytes in such proportion as in Case 1 are not found in the cerebrospinal fluid in acute lymphocytic choriomeningitis (Van Rooyen and Rhodes, 1940). This disease is known to be of virus origin, and the infection may sometimes arise as a result of a prick or abrasion (Findlay, Alcock, and Stern, 1936). The disease may also arise in small epidemics (Naville, 1931). A similar form of serous meningitis has been reported in association with serum disease (Mason, 1922) and also after inoculation of scarlet fever antitoxin (Kennedy, 1929), but here again leucocytes were not found. No description has been found in the literature of a benign meningitis having an equal admixture of lymphocytes and leucocytes in an apparently sterile cerebrospinal fluid, but the group of diseases loosely called aseptic meningitis is a large one which has not fully been explored, and may well include examples of this present type of affection.

The muscular symptoms, including spontaneous pain and soreness, exquisite tenderness on light pressure or movement, weakness, an abnormally firm texture of the affected muscles, depression of deep reflexes, hyperaesthesia and oedema of overlying skin, and especially the proximal distribution of the affected muscles, are all characteristic of a true acute myositis. This portion of the syndrome shows great similarity to epidemic myalgia (Bornholm disease) which has occasionally been recognized in this country (Williamson, 1924; Pickles, 1933). Not only is the nature of the muscular affection identical, but the distribution of the inflamed muscles is also similar, for sparing of the muscles of the extremities is usual, while involvement of the rectus abdominis, erector spinae, pectorals, and glutei have all been described (Sylvest, 1934). The present syndrome is different in the

apparent sparing of the diaphragm and in the widespread muscular involvement, which are the exception rather than the rule in Bornholm disease. The seasonal and age incidence is the same in the two conditions, and the acute febrile onset with sweating, but without respiratory signs or coryza, is similar. Pleurisy and encephalitis have also been encountered in epidemic myositis (Sylvest, 1934), although a meningeal reaction has not been described. The severe generalized muscular affection in the first case resembled rather the polymyositic form of acute toxi-infective myositis (Wilson, 1940). The syndromes grouped under this heading have so often been reviewed (Karelitz and Welt, 1932) that they need not be enlarged upon here.

A rash similar to that seen in three of these cases occurs in dermatomyositis or dermatoneuromyositis, and indeed the present illness consisted of the simultaneous onset of an acute meningeal reaction and acute dermatoneuromyositis in three cases, neuromyositis in one, and myositis in the last. The cases appeared to represent different grades of severity of the same disease. In the first the rash was a confluent erythematous and urticarial reaction, while in the second and fourth it consisted of a simple erythema. It was followed by desquamation, but was unlike scarlatina in its irregular distribution.

The tingling and numbness of the extremities, severe hyperalgesia, tenderness of the large nerve trunks, and depression of tendon reflexes all suggested an acute peripheral neuritis. These symptoms were transient and had all disappeared in a few days, by which time even the reflexes were returning. Severe polyneuritis is known to be a rare sequel of T.A.B. inoculation (Kennedy, 1929). The papillitis in Case 1 may have been evidence of a similar neuritis in the optic nerve, and the photophobia could be explained on this basis, or it may simply have been part of the meningeal syndrome. The only type of neuritis which occurs in epidemic form without an obvious dietary or toxic cause is acute infective polyneuritis (Barber, 1940), but whereas the cerebrospinal fluid had a pleocytosis and low protein in the present syndrome, it characteristically shows in acute infective polyneuritis a high protein content without a cellular increase. It has been suggested that the disease may be due to a virus infection, although all attempts to transmit it have so far failed.

To summarize, if the disease described in the present paper is considered to be due to an unknown virus, it is interesting to relate it to allied diseases of virus origin. Its many similarities to Bornholm disease have been pointed out. The meningeal part of the syndrome has some features like acute lymphocytic choriomeningitis and its rapid onset with fever, sweating, abdominal pain and tenderness, and superficial hyperaesthesia, resemble the onset of the fatal case of infection with 'B' virus (Sabin and Wright, 1934).

#### *Addendum*

Since this paper was prepared for publication a syndrome described as 'cerebral influenza simulating early cerebrospinal meningitis' has been re-

corded (Pattison, 1940). Twenty consecutive cases occurred in two military units. The features of the syndrome were a rapid onset of malaise and pyrexia, with severe headache, muscle and joint pains, signs of meningeal irritation, and in some cases photophobia and a rash. Unfortunately, a lumbar puncture was performed in one case only, so that exclusion of allied conditions is difficult. For instance, in nine of the men who had a petechial rash mild cerebrospinal fever cannot be excluded, especially as sulphapyridine was administered to some. Nevertheless several features of the two small epidemics reported closely resemble the present syndrome. The rapid onset of pyrexia, often  $101^{\circ}\text{F.}$  to  $103^{\circ}\text{F.}$ , with severe malaise unaccompanied by any inflammatory signs of the throat, nose, or chest, was characteristic of both. The distribution of the muscular pains occurring especially in the neck and back, and in two in the abdominal wall, was similar. Headache was the outstanding feature in every case. Seven men had neck rigidity, nine had photophobia, and in some a scarlatiniform rash was present. All were suspected of cerebrospinal fever, but the single sample of cerebrospinal fluid examined was clear and sterile. The cell content was not reported. Lastly, the patients all recovered uneventfully after a short illness. The only case selected for description because of its unusual features was identical with those reported in the present communication. The small local epidemics reported by Pattison (1940) seem to have appeared at the same time and in similar circumstances to that described above, and the clinical features of the cases, though unusual, were very similar to those described in the present report. It must be concluded, therefore, that a previously undescribed infection, the causal agent of which has not been isolated, occurred in this country in epidemic form in the spring of 1940.

### *Summary*

1. Five examples of a hitherto undescribed syndrome occurred at a single Royal Air Force station during the early summer of 1940. No similar cases were reported from any other station.

2. The patients were all healthy young recruits who had just arrived at a large recruiting depot where routine inoculation with tetanus toxoid and T.A.B. vaccine is undertaken. The illness began within 48 hours of inoculation with T.A.B. vaccine.

3. There was an acute febrile onset with symptoms and signs of meningitis, and myositis involving the abdomen, chest, shoulder and pelvic girdle, and the proximal portions of the limbs. Four patients had transitory symptoms of polyneuritis, three had a rash, and three had visual symptoms. The syndrome lasted for less than a week, but it was complicated by acute gastro-enteritis in one case and by a pleural effusion in another.

4. The cerebrospinal fluid was sterile, and contained excess of lymphocytes and polymorphonuclear leucocytes in three cases. All other investigations were negative.

5. The available evidence suggests that the cases represented a local epidemic of virus origin for which the T.A.B. vaccine was not responsible, though the symptoms may have been precipitated by it. The relationship of the syndrome to allied diseases is discussed.

I am grateful to the Senior Medical Officer of the Royal Air Force station concerned for supplying the early data on these cases, to the Officer Commanding Princess Mary's R.A.F. Hospital, Halton, for permission to publish the case notes, to Group Captain C. P. Symonds, R.A.F.V.R., for seeing three of the cases and giving advice upon their management, and to Air Marshal Sir Harold Whittingham and Professor G. S. Wilson for their criticism and advice upon the nature of this syndrome.

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# SIMMONDS' DISEASE OR PANHYPOPITUITARISM (ANTERIOR)

## ITS CLINICAL DIAGNOSIS BY THE COMBINED USE OF TWO OBJECTIVE TESTS<sup>1</sup>

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With Plates 16 to 18

### *Introduction*

IN 1914 Simmonds showed that a syndrome of ill-health, which he described as a 'fatal cachexia', might result from a destructive lesion of the anterior pituitary. This pathological basis has been amply confirmed in other cases with a similar syndrome (Reye, 1926; Calder, 1932; Silver, 1933; May and Robert, 1935; Sheehan, 1939, 1940). To Simmonds's original clinical description of severe panhypopituitarism (anterior)<sup>2</sup> little has since been added, but the delineation of the syndrome to include also the milder forms of the disease has proved difficult. Though Simmonds originally called it a 'fatal cachexia', in some cases it is not fatal for decades, and in some cases cachexia is not recognizable. His original case lived for 11 years after the onset of the disease, and there have been several other proved cases living for 10, 20, or more years (Fahr, 1918; Jakob, 1923; Sheehan, 1939; Means, Hertz, and Lerman, 1940). Further, although Silver (1933) concluded from his review of 41 reported cases that cachexia was the one essential clinical feature apart from hypogonadism, this has not been borne out by many reports of cases which have been examined *post mortem* (Sainton and Rathay, 1908; Fahr, 1918; Reye, 1926; Bratton and Field, 1934; Castlemann and Hertz, 1939; Sheehan 1939). In 1922 Lichtwitz denied that cachexia was an essential feature of the disease, and Sheehan (1939) after analysis of the available data, found that nutrition had usually appeared normal and sometimes there had not been even any loss of weight. Nevertheless, apart from the endocrine glands, splanchnomicria was the one feature frequently found at autopsy (Farquharson, Bell, and Duff, 1939; Silver, 1933), and when the disease had begun in early life, growth was always

<sup>1</sup> Received April 19, 1941.

<sup>2</sup> The term 'panhypopituitarism (anterior)', simplified in later references to 'panhypopituitarism', is used throughout this paper to refer to the condition of decreased anterior pituitary function involving all its hormones; it was thought possibly misleading to describe all the milder manifestations as Simmonds' disease, especially as our cases lacked final proof by autopsy.

retarded. Thus evidently a generalized atrophic process is a central feature of the disease, but clinically recognizable cachexia is often absent. Further, from his survey of the clinical picture in 51 pathologically proven cases and a review of the literature, Sheehan (1939) has been unable to find any reliable clinical sign; he concludes that certain diagnosis is possible only *post mortem*, though it may often be probable when there is evidence other than the clinical picture pointing to pituitary disease, for example a history of postpartum haemorrhage or signs of a pituitary tumour.

It is, however, of practical importance to make the diagnosis during life. Addison's disease may occasionally be mistaken for panhypopituitarism, but when the diagnosis has been narrowed down to either one of these diseases, a therapeutic test with adrenal cortex would be reasonable and safe. However, Castlemann and Hertz (1939), and later Means, Hertz, and Lerman (1940), after pointing out the close clinical resemblance which some of these cases have to myxoedema, have noted how thyroid therapy in such patients may precipitate a crisis of adrenal insufficiency. Also the close resemblance of anorexia nervosa to Simmonds' disease has been much discussed recently (Farquharson and Hyland, 1938; Ryle, Sheldon, and Spence, 1939; Rahman, Richardson, and Ripley, 1939), and while such cases often respond to correct psychiatric treatment, this could only be a wasted effort in Simmonds' disease. It is therefore specially important to be able to distinguish panhypopituitarism from anorexia nervosa and myxoedema. It is the purpose of this paper to present evidence that a reliable and objective method is now available for establishing the diagnosis of Simmonds' disease during life. During 1939 we applied to many patients with various disorders two tests which might reveal anterior pituitary defect—an insulin tolerance test and a urinary 17-ketosteroid assay. Below are presented the results of these tests applied to a series of 10 cases of Simmonds' disease or panhypopituitarism, in which the diagnosis appeared well established by various means, and also for comparison, the results obtained from 15 cases with allied syndromes or less definite pituitary defect. The latter group included three cases of myxoedema, proved to be primary, and eight cases of anorexia nervosa. An analysis has also been made of the clinical features in the 10 cases of panhypopituitarism, which reveals their close resemblance to those of Sheehan's (1939) pathologically proven cases, but for each case there was also other evidence, which is discussed later, suggesting pituitary disease.

#### *The Tests Used*

*Insulin tolerance test (I.T.T.).* A detailed study of this test is published elsewhere (Fraser, Albright, and Smith, 1941*b*), and the technical details for applying it are described in Appendix 1. For all cases suspected of panhypopituitarism the preparation of the patient, by diet, etc., is specially important, and, if a severe defect is suspected, the modified test should be used. The test consists in following the curve of blood-sugar for two hours

after a standardized intravenous injection of insulin. The test is designed to produce in normal subjects a maximal fall of blood-sugar which is reached between 20 and 30 min. after the injection; then follows a spontaneous return to the fasting level completed at least within a further 90 min., i.e. by the 120 min. sample. After the 120 min. sample the response to an intramuscular injection of adrenalin is usually measured, but when the test is used for obtaining evidence of panhypopituitarism, the adrenalin response is not very important and may be omitted, since abnormalities therein mainly indicate merely the degree of persistent insulin effects if this disorder be present. The rate of the initial fall in the blood-sugar differentiates normal or increased insulin sensitivity from insulin resistance; and the speed of the subsequent return to the fasting level will indicate any tendency to persistence of hypoglycaemia, that is, a delay in return will indicate 'hypoglycaemia unresponsiveness'<sup>3</sup> which may be found mainly in hyperinsulinism, hypoadrenocortinism, or panhypopituitarism. Thus the characteristic result of this test in panhypopituitarism is a normal rate of fall associated with an abnormally slow return to the fasting level, or 'hypoglycaemia unresponsiveness'. Blood-sugars were estimated by the method of Folin and Wu (1934) on non-laked capillary blood.

*Urinary 17-ketosteroid assay.* This assay was done on a 16- or 24-hour specimen according to the method of Callow (1938), after carbon tetrachloride extraction (Callow, 1940); fuller details of the method and the results obtained in other conditions are given elsewhere (Fraser, Forbes, Albright, Sulkowitch, and Reifstein, 1941 *a*). It measures a group of steroids whose output is probably an index of the total steroid excretion from both the adrenal cortex and the male gonad. Thus it offers an indirect measure of anterior pituitary function, but it will be seen that it should be expected to differentiate Addison's disease from panhypopituitarism only in male subjects.

### Results

*Panhypopituitary (anterior) cases. Insulin tolerance test.* In all cases the test gave a characteristic curve; after a normal initial fall of blood-sugar there was a failure or delay in the usual spontaneous return (see Fig. 1 and Table II). The adrenalin response was usually abnormally slight, but varied mostly in accordance with the degree of hypoglycaemia unresponsiveness. The hypoglycaemia unresponsiveness may be expressed quantitatively by measuring the slope of the curve after the maximal fall has been reached. It may be done most simply by using the figure of the 120 min. blood-sugar expressed as a percentage of the fasting level. Since normally it is never under  $100 \pm 10$  and is usually  $105 \pm 5$  or over, the ratio is already as a percentage of the normal, and results under 90 per cent. are of significant abnormality; in our cases it varied between 80 and 47 per cent., with a

<sup>3</sup> 'Hypoglycaemia unresponsiveness' has been suggested as preferable to insulin hypersensitivity for describing this abnormality.

mean value of 63 per cent. The slope may be more accurately measured by an index<sup>4</sup> taking into consideration all the points on the curve after the maximal fall has been reached; this 'index of hypoglycaemia responsiveness' was found to range in normal subjects from 474 to 621, with a mean about 550. With our 10 panhypopituitary cases it varied between 194 and 395, with a mean of 330. It may be noted that in some of the cases the blood-sugar, after reaching its maximal fall, did not rise at all until the adrenalin

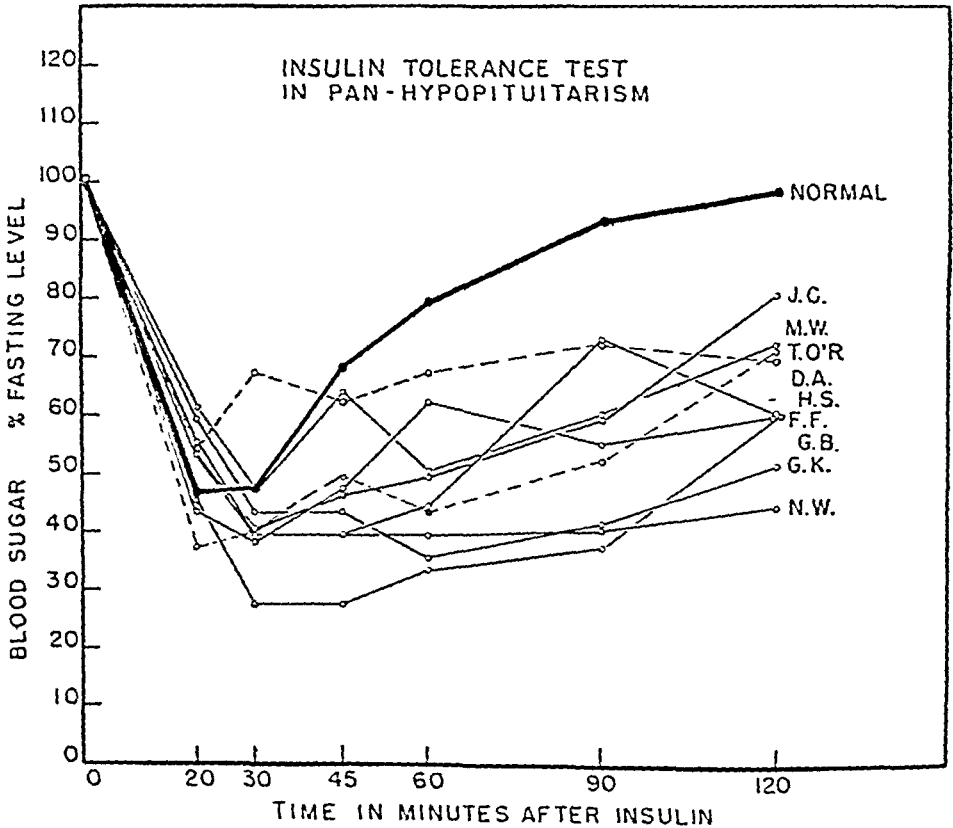


FIG. 1. *Insulin tolerance test results on panhypopituitary patients.* The average normal curve is shown for comparison. Details of these tests are given in Table II. It will be noted that the initial fall of blood-sugar corresponds to the normal, but that the subsequent return is abnormally delayed, i.e. 'hypoglycaemia unresponsiveness' is present.

injection at the end. To a certain extent it appeared that the degree of failure in the return of the blood-sugar corresponded to the degree of pituitary failure as assessed by clinical signs. As previously mentioned, similar abnormal insulin tolerance curves may be found with hyperinsulinism, Addison's

<sup>4</sup> This index is calculated by multiplying the blood-sugar value (expressed as a percentage of the fasting level) of the 45, 60, 90, and 120 min. samples by the time ( $\frac{1}{2}$  hour = 1) after the previous sample, and then adding all these together. It is a close approximation to the area enclosed between this curve and the zero blood-sugar line. It is, therefore, an inverse measure of the degree and persistence of hypoglycaemia after the 30 min. sample or reaching the maximal fall; it is thus an index of 'anti-hypoglycaemia' function.



disease, and possibly with severe liver disease or malnutrition, but it should be possible to differentiate these conditions either clinically or by the other test.

*Urinary 17-ketosteroid assay.* With one exception (Case 4, probably the mildest case), all these have been zero<sup>5</sup> (under 0.5 mg. per 24 hours). Each case had at least two assays, and the majority six or more. The assays in Case 4 were 1.3 and 1.5 mg. per 24 hours, which is well below the normal range (women average 9.0 mg., range 5 to 15 mg.; men about 4 mg. higher). This striking decrease to zero is, however, found also in other conditions, notably in cirrhosis of the liver, steatorrhoea, female Addison's disease, and myxoedema (Fraser, Forbes, Albright, Sulkowitch, and Reifenstein, 1941 a). The excretion may also be lowered, but not to zero, in male hypogonadism, male Addison's disease, and general malnutrition. As mentioned later, these conditions may probably be distinguished either clinically or by the previous test. It will be noted that malnutrition also lowers the excretion. It has been a general experience that where wasting was a feature in a case of Simmonds' disease, it indicated either severe pituitary disease or complicating defective intake of food. Therefore, although our mildest case gave a low positive assay, it would be extremely unlikely that a seriously wasted case of Simmonds' disease would not have the usual zero assay. This point is significant in relation to the findings with anorexia nervosa mentioned later.

*Clinical picture.* An analysis of some of the more important clinical features of the cases is shown in Table I, together with the findings of the two special tests; the histories are given in fuller detail in Appendix 2.

In these cases, all still alive, the disease had already been present for periods varying from 5 to at least 18 and probably 45 years. Most of the patients still continued with some form of light work, such as housework, cooking, school, &c. Apart from those symptoms attributable to hypogonadism (amenorrhoea or eunuchoidism), practically the only universal symptom was a loss of energy and alertness, having no specific features, but seeming to be more or less proportional to the severity and duration of the lesion. Excepting only the slighter cases, it has been associated with some degree of anorexia, constipation, and a tendency to depression and irritability. When the condition had originated before adult life there was also slowing of somatic growth; slow growth is evidently still proceeding in all these patients, probably including even one aged 45 years. Intellectual development is evidently affected little, if at all, as shown by the satisfactory school progress of the pituitary dwarfs studied. In some cases (e.g. Case 2), after the disease has been present for many years, mental alertness seems to fail and the patient becomes dull, slowed, somnolent, and may show forgetfulness; possibly this may be due to an abnormally early cerebral deterioration or more probably to the later development of complete thyroid failure.

<sup>5</sup> It should be noted that a colorimetric correction for interfering colours was used; this transformed some otherwise low positive assays to zero (Fraser, Forbes, Albright, Sulkowitch, and Reifenstein, 1941 a).

TABLE I. *Clinical Features and Test Results of 10 Cases of Panhypopituitarism*

This summarizes the findings in the panhypopituitary patients which are discussed in the text. The urinary follicle stimulating hormone tests were done on concentrated morning urine according to the method of Lovin and Tyndall (1937); the details of the insulin tolerance test results on these patients are shown in Fig. 1 and Table II; the case histories are given in detail in Appendix II.

Case	Duration	Skin	Adipose tissue	Nutrition	B.P.	Other clinical features	B.M.R. %, choles- terol mg. %	Urine- ary F.S.H.	Therapeutic response	Insulin toler- ance test	17-keto- steroids	Probable aetiology
1. H.S. Female	7 years, since age 23	Clear, thin, sallow, and dry	None	Normal	90/72	Dyspnoea on exertion, headaches and fainting turns, oedema, and anaemia	-35 to -45	Neg. (2.5 m.u.)	Addisonian crisis after thyroid; men- strual period after follicle- stimulating hormone	HgI. unresp. (120 min. = 01 %) H.R.I. = 355	0.0 per 24 hr. (0 assays)	Severe post partum haemorrhage
2. N.W. Female	17 years, since age 37	Clear, pallid, atrophic, and dry	Very sparse	Normal, but slight loss of weight	95/60	Dyspnoea on exertion, oedema, lethargy, ano- rexia, constipation, and anaemia	-37 to -43	Neg. (5 m.u.)	—	HgI. unresp. (120 min. = 60 %) H.R.I. = 244	0.0 per 24 hr. (3 assays)	"
3. M.W. Female	9 years, since age 25	Thin and sallow, but moist	None	Normal	—	Very slight asthenia	-23	Neg. (10 m.u.)	—	HgI. unresp. (120 min. = 73 %) H.R.I. = 14	0.0 per 24 hr. (3 assays)	"
4. G.B. Female	5 years, since age 25	Thin and pale	None	Normal	—	Very slight asthenia	-4 to -9	Neg. (10 m.u.)	—	HgI. unresp. (120 min. = 67 %) H.R.I. = 300	1.3 and 1.5 per 24 hr.	"
5. F.F. Female	15 years, since age 22	Thin, slightly dry, sallow, atrophic	None	Normal, but slight loss of weight	80/60	Moderate anergia, ano- rexia, fainting turns, headaches, and anaemia	-31 to -37	Neg. (2.5 m.u.)	Positive with thyrotrophic hormone	HgI. unresp. (120 min. = 47 %) H.R.I. = 240	0.0 per 24 hr. (0 assays)	Chromophobe pituitary tumour; relieved by X-ray; complicated by pancreatic diabetes
6. T.O.R. Male	8 years, since age 11	Thin, slightly dry, and atrophic	None	Low average	70/40	Moderate asthenia, dwarfed with 'bone age' of 12, jagged facies, fainting turns, one con- vulsion, and anaemia.	-30	Neg. (10 m.u.)	Positive with thyrotrophic hormone; with chlorotic gonadotrophin	HgI. unresp. (120 min. = 72 %) H.R.I. = 354	0.0 per 24 hr. (0 assays)	Suprarenal cyst, re- moved by operation
7. G.H. Female	1 since birth (45 years)	Thin, sallow, and slightly dry	None	Normal	120/90	Very slight asthenia, 'bone age' under 18, no anaemia	—	Neg. (10 m.u.)	—	HgI. unresp. (120 min. = 61 %) H.R.I. = 345	0.0 per 24 hr. (3 assays)	?
8. D.A. Male	1 since birth (10 years)	Thin	None	Normal	80/60	Slight asthenia, dys- pnoea, 'bone age' under 10, and anaemia	-13 to -20	Neg. (10 m.u.)	—	HgI. unresp. (120 min. = 70 %) H.R.I. = 302	0.0 per 24 hr. (0 assays)	?
9. J.C. Female	1 since birth (21 years)	Thin, sallow, and slightly dry	None	Normal	80/60	Anorexia, dyspnoea, moderate asthenia, dwarfed with 'bone age' of under 12, and anaemia	-33 -32	Neg. (10 m.u.)	—	HgI. unresp. (120 min. = 80 %) H.R.I. = 380	0.0 per 24 hr. (0 assays)	? Following neonatorum
10. G.K. Male	18 years, since age 35	Dry, sallow, and atrophic	None	Normal	155/80	Dyspnoea on exertion, oedema, dizzy spells, Addisonian crisis after a cold, anaemia, and acanthrohyria	-27 to -30	Neg. (10 m.u.)	Positive with thyrotrophic hormone; with chlorotic gonadotrophin	HgI. unresp. (120 min. = 52 %) H.R.I. = 268	0.0 per 24 hr. (0 assays)	?

Loss of weight of more than a few pounds had occurred in only one of our cases, but even this patient did not look emaciated. Evidence of what was probably a generalized atrophy was found in the state of the skin and hair, the only directly accessible tissues. The skin in most cases was thin, inelastic, dry, and of a pale sallow colour, usually looking excessively smooth. In the least marked cases it was pallid, thin, and delicate by comparison with that of the patient's relatives, but showed no other abnormalities. Sheehan (1939) has pointed out two modifications in the skin condition that may be seen—the characteristic dry wrinkled atrophy in progeria, the severe form of the disease, and that seen in cases where associated myxoedematous features are marked, a dry scaly skin associated with diffuse subcutaneous thickenings. Some of our cases showed the latter type (e.g. Case 2). There was also in all our cases considerable decrease or more usually complete loss of axillary hair; with the severer cases this also involved body, beard, and pubic hair. The scalp hair was usually rather fragile; in one patient (Case 5) it had turned grey at the age of 23 years. Sheehan (1939) pointed out that this loss of hair is gradual, becoming obvious within a few months, but is rarely complete in less than five years, and affects the labia least of all.

There were many other signs seen in severer cases, none of which were universal, but which complete the picture of generalized atrophy shown in the fully developed form described by Simmonds. There was often evidence of circulatory inefficiency—cold extremities and low blood-pressure, and in the more advanced cases dyspnoea on exertion. Some of our patients have shown slight oedema, affecting particularly the face and feet, which may be associated with the thyroid failure. Five of our 10 cases have had 'turns' of various sorts which were probably attributable to hypoglycaemia (attacks of faintness, headaches, dizzy spells, and even convulsions).

There was an anaemia present in most of the cases which were tested; as pointed out by Sheehan (1939) the minimal degree appears to be a fall of haemoglobin percentage with, in the moderate cases, an associated fall in the red cell count, but in the more advanced cases the red cell count falls till the colour index rises to just over 1.0. In some of our cases where they were tried, iron or liver extract therapy did not improve the anaemia. We have not studied gastric secretion in many cases, but Sheehan (1939) pointed out that there is a hypochlorhydria in about half the cases. The basal metabolic rate has been found lowered in all cases, usually to about -30 per cent. (limits -20 per cent. (? -13 per cent.) to -45 per cent.). Sheehan stated that it usually remains about -30 per cent. for the first 15 years of the disease, but often falls after this to the region of -40 per cent., probably due to the development of an associated myxoedema. Where the disease had commenced before maturity, the slowing of somatic growth was confirmed by epiphyseal X-rays. In none of these cases, including the one aged 45 years, was epiphyseal union complete. The serum-cholesterol was usually increased, particularly in the milder cases; in some cases it was normal, and in the two severest cases it was diminished (see Table I).

TABLE II. *Insulin Tolerance Test. Panhypopituitarism: Blood-sugar Values as Percentage of Fasting Level*

In the first two columns are given the preliminary diet preparation and the dosage of insulin used; it should be noted that some of the tests were done by the modified procedure using less than the standard dose of insulin. In the column 'Symptoms' is shown the duration and severity of obvious hypoglycaemic symptoms and signs. In the column H.R.I. after the figures for blood-sugar samples are given those for the 'hypoglycaemia responsiveness index' of each test; normal values for this are  $550 \pm 70$ . In some instances, for various reasons, the index is not valid for comparison with the normal figures. Where less than the standard dose of insulin was used, the H.R.I. figure is given in brackets, but it will be seen to be usually still abnormally low. For the second test on Case 6 (this figure is marked †) the test had been given after a low preliminary carbohydrate diet instead of the usual diet in order to illustrate the importance of this preparation (cf. the previous test on this patient). For the second test on Case 1 an estimate for the 120-min. sample was necessary in order to calculate an index, as it had been necessary to inject adrenalin and interrupt the test after the 90-min. sample, since unduly severe hypoglycaemic symptoms had supervened (this might have been avoided by giving a modified test). The 90-min. figure was used for the 120-min. value in calculating this index. It should be noted that the third test on Case 5, was done after improvement following X-ray treatment; the other two tests on this patient are the only comparable tests on a single patient, and the similarity of the results may be noted.

Case and date	Diet, gm. carbhydrate per diem	Insulin units	Blood-sugar values (as percentage of fasting level)													Symptoms	H.R.I.
			Minutes after insulin									After adrenalin					
			0 (fasting level)	20	30	45	60	90	120	30	45	60					
1. H.S. 16/4/39 15/6/39	3 days 350 Poor	5.0 (Std.) 5.0 (Std.)	100 (80) (100) (52)	54 (80) (100)	40 (80) (100)	40 (80) (100)	45 (80) (100)	74 (80) (100)	61 (80) (100)	— (80) (100)	— (80) (100)	— (80) (100)	— (80) (100)	— (80) (100)	20-60 min. severe; 60-120 min. less so; 20-60 min. moderate; 60-30 min. after adrenalin, severe confusion	355 286	
2. N.W. V. Age 54 1/12/39 No. 223908	200	4.6 (Std.)	100 (62)	56	40	40	40	42	50	—	53	108	—	—	20-60 min. severe; 60-120 min. moderate	244	
3. M.W. 20/4/39	6 days 350	5.6 (Std.)	100 (73)	62	48 v 48 v	65 v 39	51 v 39	61 v 60	75 v 60	—	—	—	—	—	20-60 min. severe	414	
4. G.B. 23/11/39	—	5.0 (Std.)	100 (70)	43	43	41	43	71	67	—	125	142	—	—	—	360	
27/11/39	—	1.6 ( $\frac{1}{3}$ std.)	100 (70)	41	63	41	43	70	—	—	149	127	—	—	—	—	

5. F.F. 22/10/38	130	6.0 (Std.)	100 (74)	60	29	28	48	35	47	—	—	—	25-90 min. moderate severity; 90-120 min. less so	240
29/11/38	130	6.0	100 (80)	46	28	28	34	38	61	—	—	—	The same	260
8/3/39 (after treat- ment)	130	6.0	100 (73)	67	55	57	57	61	79	—	—	—	The same	394
6. T.O.R. 3/12/38	4 days 250	3.5 (Std.)	100 (50)	38	40	50	44	58	72	—	—	—	20-60 min. severe; 60-80 min. moderate	354
10/12/38	4 days 50	3.5 (Std.)	100 (51)	51	27	35	71	69	99	—	—	—	20-60 min. moderate	442†
7. G.B.	4 days 350	3.6 (Std.)	100 (72)	44	39	48	63	56	61	—	—	—	20-60 min. severe; 60-100 min. less so	345
8. D.A. 6/7/39	—	1.2 ( $\frac{1}{3}$ std.)	100 (60)	55	66	73	83	87	71	—	—	92	30-45 min. slight	(472)
21/7/39	—	1.6 ( $\frac{1}{2}$ std.)	100 (78)	53	68	63	68	73	70	98	—	110	30-35 min. slight	(417)
10/8/39	—	2.4 ( $\frac{2}{3}$ std.)	100 (78)	60	55	47	57	—	47	—	—	—	None	(302)
9. J.C. 10/8/39	Ordinary	2.0 ( $\frac{1}{2}$ std.)	100 (48)	54	42	48	50	60	81	—	—	—	20-45 min. severe; 45-90 min. less so	(380)
10. G.K. 6/4/39	5 days	6.5 (Std.)	100 (86)	60	44	44	36	42	52	—	60	—	20-120 min. severe	(268)

*The allied or less definite cases studied* (Table III). There is insufficient space for the inclusion of the results of these tests on all the allied disorders studied; the data and a closer analysis of the tests have been published elsewhere (Fraser, Forbes, Albright, Sulkowitch, and Reifenshtein, 1941 *a*; Fraser, Albright, and Smith, 1941 *b*). However, it is probably important to present here the results with the disorders which it is especially difficult to differentiate clinically from panhypopituitarism, that is, primary myxoedema, parapituitary tumours, anorexia nervosa, and dwarfism from other causes. All the cases of myxoedema have subsequently been completely cured by thyroid medication; the parapituitary tumour cases were either those in which there were no clinical signs of pituitary involvement, or in which the extent of this was difficult to assess clinically; and the cases of anorexia nervosa had the characteristic psychiatric and other clinical features of this condition.

Case C. B., an adolescent youth of 19 years, was probably a pituitary dwarf, but the tests failed to reveal conclusive evidence of pituitary defect, the insulin tolerance test giving normal results, and the 17-ketosteroid assay being abnormally low, but not zero. There were no symptoms apart from dwarfism, so at the most there was only very slight panhypopituitarism. The cases of parapituitary tumour illustrate various points. Case 15 had X-ray signs of a suprasellar tumour, but no symptoms other than visual failure due to optic atrophy. In his case both the tests gave normal values. Case 16 had signs indicating a tumour involving the third ventricle and suggesting also the possibility of pituitary involvement; later at autopsy this was proved to be the case. In his case the tests indicated pituitary defect; the 17-ketosteroid assay was zero, and the insulin tolerance test was just on the limit of normal (Table III). Cases 17 and 18 were shown by X-ray and subsequent operation to have suprasellar cysts pressing on the pituitary. In both cases the 17-ketosteroid assay was zero before operation, but some months later, when there were still signs indicating hypothalamic damage, though no symptoms of panhypopituitarism, the assays were in the lower region of normal (5 to 6.5 mg. per 24 hours), confirming the clinical impression of improved pituitary function.

Three cases of primary myxoedema were studied by both tests. The 17-ketosteroid assays were all very low, varying between zero and 2.0 mg. per 24 hours, indicating that this test cannot serve to distinguish primary hypothyroidism from panhypopituitarism. The results indicate that insulin tolerance tests can differentiate them, and with the myxoedema patients this test showed a slow initial fall or insulin resistance; the maximal fall was reached usually at the 45 min. sample, instead of between 20 and 30 min., and the hypoglycaemic symptoms were either absent or delayed till well after the 30 min. sample, features not found with either normal or panhypopituitary cases. Although the 120 min. sample with these cases is often abnormally low (76 to 100 per cent.), this figure alone is not an index of the hypoglycaemia responsiveness, since the initial fall was delayed. It

may be noted that insulin tolerance test results from the cases of panhypopituitarism (e.g. Cases 1 and 10) which showed clinical and basal metabolic rate evidence of the co-existence of complete myxoedema, were still typical of panhypopituitarism; evidently the effect of panhypopituitarism on this curve predominates over that of myxoedema. Thyroid treatment had failed to relieve the symptoms of either of these patients, in contrast to the primary myxoedema cases. Thus, whether or not a moderate degree of hypoglycaemia unresponsiveness be present with the myxoedema cases, the evidence of insulin resistance is adequate to exclude any significant degree of panhypopituitarism. Elsewhere we have reported other studies of carbohydrate metabolism in both these disorders (Fraser, Albright, and Smith, 1941 *b*); other tolerance tests corroborate these interpretations of the insulin tolerance test in myxoedema and its ability to differentiate the two conditions.

Eight cases of anorexia nervosa were studied and either one or both of the two tests performed. With the insulin tolerance test some, but not all, gave normal results, and so might have been differentiated from panhypopituitarism by its use. Of the eight cases, all of which showed a considerable degree of wasting, four gave normal results, one gave a result just outside the normal limits, and two gave definite evidence of hypoglycaemia unresponsiveness. The preparation by diet had been doubtful in these latter cases. The remaining case (No. 28) gave an inconclusive result, because it had been impossible to give an adequate preliminary carbohydrate intake. The maximal fall was not reached till 60 min., and the return to the fasting level not completed by 120 min. As was shown initially by Himsworth (1935), a low carbohydrate intake induces insulin resistance. In all six cases in which a second test was carried out, which included all those (except No. 28) giving abnormal insulin tolerance results, 17-ketosteroid assays were found positive, varying between 2.7 and 14.7 mg. per 24 hours; the majority were just within the normal range. The other cases, unfortunately, were not tested. All cases of anorexia nervosa have lost considerable weight; as already pointed out, it is most unlikely that a case of cachexia due to panhypopituitarism would give a positive test, however low, since malnutrition both lowers the assay itself and would usually indicate severe disorder. Thus it is suggested that positive assays, apparently always present in anorexia nervosa, distinguish this disorder from Simmonds' disease. It remains, however, a possibility that a slightly lowered pituitary function may coexist in anorexia nervosa, very probably caused by the malnutrition. This could explain both the lowering of the 17-ketosteroid assay and the occasional abnormalities in the insulin tolerance test, but there is no justification for such a conclusion, since these changes may both be due to other metabolic disorders consequent on the malnutrition, possibly liver dysfunction, for example. While it appears that a positive assay in a severely wasted patient should exclude panhypopituitarism, unfortunately the converse cannot hold till a large series of wasted patients has been tested; from our findings it seems probable that at least in the majority of instances it would.

TABLE III. *Insulin Tolerance Test and Urinary 17-ketosteroid Assays in Various Allied Conditions*

The insulin tolerance test results are shown as blood-sugar values in the first column, following which in the H.R.I. column are given the hypoglycaemia responsiveness indices calculated from these according to the method described in the text (normal values  $550 \pm 70$ ). No figure is given in this column for the myxoedema cases or Case 28, where the initial fall was so delayed as to make this figure not comparable with normal subjects. The characteristic slow and prolonged initial fall will be noted in the insulin tolerance tests of the myxoedema cases, while the majority of the other insulin tolerance test results are normal, or nearly so. The low 17-ketosteroid assays of the myxoedema cases will be noted, and also the universally positive and often normal 17-ketosteroid assays of the other cases.

Case and date	Blood-sugar values (as percentage of fasting level)								H.R.I.	17-ketosteroid assay (mg. per 24 hr.)	Diagnosis	
	0 (fasting level)	20	30	45	60	90	120	Primary oedema)			hypothyroidism (myxo-	
11. H.C. 2/10/32	100 (87)	92	82	61	43	84	76	—	1.0, 0.7, and 0.0			
12. M.B.	100	79	67	50	33	84	82	—	1.5, 1.3, and 1.5	"	"	
13. M.H. 30/1/40	100 (77)	55	45	44	60	64	90	—	0.9, 1.1, 1.7, and 1.2	"	"	
15. B. 29/12/39	100 (84)	66	50	60	75	91	95	507	9.5	Suprasellar tumour producing optic atrophy; no clinical signs of pituitary involvement		
16. J.L. 15/12/30	100 (61)	61	62	69	70	85	90	489	0.0 (3 assays)	Brain-stem tumour spreading into pituitary		
17. M.G. 6/2/40	100 (83)	48	58	84	89	81	80	495	5.0 and 5.5	Suprasellar cyst, three months after operation		
18. M.R. 17/11/39	100 (63)	76	60	51	65	76	108	484	6.0 and 6.5	Suprasellar cyst, three months after operation with symptoms indicating hypothalamic damage		



		87	44	61	69	83	97	490	5.9, 6.8, and 9.6	Anorexia nervosa
21. G.H. 19/6/39	100 (82)									
22. M.E. 6/9/37	100 (35)	58	29	61	44	88	109	519	—	”
23. M.P.	100 (84)	79	77	69	77	99	110	564	—	”
24. M.P. 12/11/36	100 (70)	67	76	86	93	103	139	603	—	”
25. A.V. 19/1/40	100 (72)	58	60	94	86	97	(?) 82	538	7.5 and 8.9	”
26. M.C. 8/2/39	100 (82)	60	44	59	60	58	73	381	2.7	”
27. H.L. 30/1/40	100 (63)	49	44	63	57	75	76	422	14.3 and 14.7	”
28. J.F. 19/8/36	100 (80)	94	94	—	75	78	80	—	—	”

*Conclusions concerning criteria for the diagnostic use of the tests in relation to panhypopituitarism.* Zero 17-ketosteroid assays (under 1 mg. per 24 hours) probably may be taken as evidence of panhypopituitarism, provided severe liver disease, steatorrhoea, Addison's disease, and hypothyroidism can be excluded. If considerable wasting is present low assays will always be found, but a positive assay probably excludes primary panhypopituitarism of a clinically significant degree. Abnormally low but positive assays should be used only as corroborative evidence, and then only provided that there is little malnutrition. With the insulin tolerance test a finding of hypoglycaemia unresponsiveness, only if associated with a normal initial fall, probably indicates panhypopituitarism, provided that Addison's disease, hyperinsulinism, severe liver disease, and wasting can be excluded. Most of the patients with wasting appear to give a normal result after adequate diet preparation. Hypothyroidism can be reliably differentiated only by the insulin tolerance test. A normal result with either or both tests is probably strong evidence against panhypopituitarism of any clinical significance; conversely, typical abnormalities in both tests probably indicate panhypopituitarism, if certain clinically distinguishable diseases are absent (Addison's disease and severe liver disease being the two important possibilities to be excluded).

### *Discussion*

The object of this paper is to show that these two tests may be used clinically for the demonstration of panhypopituitarism. They are based on well recognized physiological principles, but it may be objected that the diagnosis in the cases studied still awaits proof by post-mortem examination. Two of the patients had X-ray or surgical evidence of a pituitary tumour, four had symptoms dating from severe post partum haemorrhage, which Sheehan (1939) has shown to be so frequently the cause of pituitary necrosis, and in three there was also dwarfism with delayed bone development. With the remaining case and with three of the others, temporary restoration of gonad or thyroid function by pituitary-like stimulation has been demonstrated. Further, analysis of the symptoms and signs in all these cases indicates that the syndrome was precisely similar to that described by Sheehan (1939) in other non-cachectic patients proved by post-mortem examination to have pituitary disease. This author has already pointed out the indefiniteness of the clinical features of the syndrome, and the inadequacy of other methods of clinical diagnosis. A suspicion of the syndrome is probably warranted in all cases of hypogonadism combined with asthenia, but the only clinical signs that appear to be universal in adults are decrease of axillary hair with generalized atrophy and pallor of the skin. Both these signs may, however, occur in other cachexias and may be difficult to establish in slight cases. The urinary follicle stimulating hormone test was negative in all cases examined. In suspected hypogonadal cases a positive follicle stimulating hormone test might validly exclude panhypo-

pituitarism, but unfortunately this test is insufficiently sensitive to be consistently positive with normal urine, and so cannot be used to prove pituitary failure.

No new physiological assumptions are necessary in order to regard the defects found by these two tests as direct consequences of panhypopituitarism. Adrenal cortical and gonadal atrophy are well recognized pathological features of pituitary defect, and available data offer reasonable proof that the 17-ketosteroids come only from the adrenal cortex, the male gonads, and just possibly the female gonads (Callow, 1939; Fraser, Forbes, Albright, Sulkowitch, and Reifstein, 1941 *a*). An intravenous insulin test has for some years been used in animals for demonstrating the effects of hypophysectomy (Russell, 1938), and the type of curve is exactly similar to that found with our panhypopituitary patients. There are several clinical reports of the occurrence of spontaneous hypoglycaemia or hypersensitivity to insulin in panhypopituitary patients (Jakob, 1923; Cushing and Davidoff, 1927; Pribram, 1927; Meng, 1928; Wilder, 1930, 1931). There are a few reports of insulin tests applied to such patients and showing decreased insulin tolerance (Lücke, 1932; Sendrail, 1930; Meythaler and Schroff, 1935). The reasons for preferring the insulin tolerance test to all other available carbohydrate tests for demonstrating the defective metabolism in panhypopituitarism have been discussed elsewhere (Fraser, Albright, and Smith, 1941 *b*). In the absence of significant non-endocrine disease, this test measures most directly, and hence probably most sensitively and accurately, the combined efficiency of those hormones which can raise the blood-sugar, i.e. mainly 'diabetogenic' pituitary and adrenal cortex activity, because the induced stress is their direct antagonist. In the same way the glucose tolerance test probably gives the best measure of insulin deficiency, but is poorly adapted to measuring pituitary defect. It is felt that the combined insulin and glucose tolerance test (Himsworth, 1935, 1936) is not so reliable, because of the possibility of defective intestinal absorption giving similar abnormal results, and as it merely measures insulin sensitivity.

It may therefore be assumed that these tests demonstrate defects characteristic of hypopituitarism, but it is unwise to attempt their interpretation without full preliminary clinical examination; in particular, infections other than focal, or severe liver disease, may vitiate the interpretation of either test, and should be excluded. The allied conditions presented in this paper have included probably the main disorders which may be confused clinically. Thus myxoedema, while giving in some cases indistinguishable results by the 17-ketosteroid assay, gives a characteristically different insulin tolerance curve. Therefore, by the use of this test, no case of primary myxoedema should be mistaken for panhypopituitarism. A raised serum-cholesterol level was found in many of the panhypopituitary cases, and so this feature of myxoedema cannot be used to differentiate primary myxoedema from panhypopituitarism, but Scowen (1937) has described a satisfactory method for making this distinction between the primary and secondary or pituitary

hypothyroidism. He has shown that the latter, but not the former, will respond to thyrotrophic hormone injections by a rise in basal metabolic rate. Since normal subjects as well as other patients with lowered basal rates also respond with a rise, unfortunately a lowered basal metabolic rate associated with this response cannot be used as evidence of panhypopituitarism.

The other disorder most readily confused, anorexia nervosa, may occasionally give indistinguishable results with the insulin tolerance test, but always gives a positive 17-ketosteroid assay. As already pointed out, such a result in a patient who has lost much weight may be taken to exclude panhypopituitarism as the primary cause. Some cases of parapituitary tumour have shown that, even in the presence of hypothalamic damage, these tests are still valid; they may indicate the absence of any significant panhypopituitary defect, or may still demonstrate this disorder when it is also present. Further work is necessary to clarify fully the effects of hypothalamic damage on the test results. Hyperinsulinism with the insulin test, and Addison's disease in women with both tests, may give results which are indistinguishable from those of panhypopituitarism. However, the former may probably be distinguished by a normal 17-ketosteroid assay, and both probably by clinical criteria. Thus it would appear that provided that both tests are performed in doubtful cases, and that adequate clinical examination is made to exclude significant non-endocrine disease, the demonstration of panhypopituitarism by these tests should be specific.

There are insufficient data for any precise conclusions concerning the sensitivity of the tests, but probably in none of our cases should the disorder be classed as severe. The disease had existed for over five years in all cases, and over 10 years in most; none looked wasted, and the majority had not lost weight. In some the symptoms produced extremely little interference with their life or ability to work. It may therefore be anticipated that pituitary defects which are not demonstrable by these tests do not produce symptoms of Simmonds' disease. The indeterminate results with one case of dwarfism, possibly of pituitary origin, suggest that the tests may not be sufficiently sensitive when dwarfism is the only sign, but it should perhaps be noted that the criteria of normality are more difficult to establish with dwarfed patients. It is therefore submitted that two tests, applied together and after proper clinical examination, are sufficiently specific and sensitive for practical application.

### *Summary*

1. Two tests, an insulin tolerance test and a urinary 17-ketosteroid assay, were applied to 10 patients believed to be suffering from panhypopituitarism (anterior) or Simmonds' disease, and to 15 other patients suffering from allied disorders.

2. An analysis is presented of the clinical signs and symptoms of the 10 panhypopituitary patients. The only universal symptoms were those of

hypogonadism and asthenia; the only universal signs, apart from those of hypogonadism, were some atrophy of the skin and loss of most or all axillary hair. The basal metabolic rate was low, but the serum-cholesterol often high.

3. With the insulin tolerance test the panhypopituitary cases showed a normal initial fall and 'hypoglycaemia unresponsiveness' or a delay in the subsequent return to the fasting level of blood-sugar. It is pointed out that this may occasionally be found in some clinically distinguishable disorders.

4. With eight cases of anorexia nervosa it was found that four gave a normal result with the insulin tolerance test, but that in three of the remainder it was similar to that found in panhypopituitarism. With three cases of primary myxoedema this test showed a slow initial fall or insulin resistance, which differentiated them from all the cases of panhypopituitarism.

5. The 17-ketosteroid assays on the panhypopituitary patients were zero (under 0.5 mg. per 24 hours) with one exception, the mildest case, in which it was reduced to under 2.0 mg. per 24 hours, a figure well below normal levels.

6. Four cases of anorexia nervosa were tested similarly. All gave positive assays ranging from 2.7 to 14.7 mg. per 24 hours, and included all those giving results with the insulin tolerance tests which were not distinguishable from panhypopituitarism. The assays in the primary myxoedema cases varied from zero to 1.7 mg. per 24 hours.

7. It is concluded that by the combined use of the tests, panhypopituitarism (anterior), producing even fairly mild symptoms, can be demonstrated and distinguished from allied syndromes. The insulin tolerance test distinguishes primary myxoedema, and anorexia nervosa is distinguished sometimes by both, and, in all cases tested, by the 17-ketosteroid assay. It is pointed out that clinical examination is also needed to exclude certain disorders, notably Addison's disease and severe liver disease.

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## APPENDIX I

### *Method of the Insulin Tolerance Test*

The preparation of the patient is similar to that required for all carbohydrate tolerance tests. For at least four days, longer if severe malnutrition has existed, the patient is given a diet containing 250 to 400 gm. of carbohydrate per diem, as well as its other constituents. It is helpful to estimate

the fasting blood-sugar on the day before any tests, and if this is within normal limits (60 to 105 mg. per 100 c.c. for the Folin Wu unlaked blood method), precisely 12 hours' fast is arranged to precede the test, that is, the patient is usually asked to have a glass of milk at 9 p.m. for a 9 a.m. test, and he should lie down for the half-hour previous to the insulin injection. If the fasting blood-sugar lies outside normal limits, the test is inapplicable in the usual way. If the fasting level is already below normal limits, this of itself indicates a defect which will probably show hypoglycaemia unresponsiveness by the test. However, the test may still be done after a shorter fast—three hours after tea and toast will usually be satisfactory if the blood-sugar is then within normal limits. While this modification might conceivably mask a defect which would cause hypoglycaemia unresponsiveness, this is unlikely and it should not itself cause an abnormal curve. If the fasting blood-sugar is found to be above the normal range the insulin tolerance test is usually inapplicable, and insulin sensitivity is best measured by the combined glucose and insulin tolerance test of Himsworth (1936).

*Standard insulin tolerance test.* After taking duplicate fasting samples of blood, 0.1 units of insulin per kg. of body-weight are given intravenously. In our cases, if the patient was grossly overweight or underweight, the dose was calculated from the average of the patient's ideal and actual weight. It might be preferable with these patients, and possibly with all patients, to give a dose based on surface area—3.7 units per sq. m. of body-surface corresponds to the dosage we have used. This figure was calculated from tables of average height and weight (extremes for ages 20 to 25 years and height 5 ft. to 6 ft. inclusive, gave 3.6 and 3.8 units per sq. m., and the ranges were not much greater up to the age of 45 years). Subsequent capillary blood-sugar samples are taken at 20, 30, 45, 60, 90, and 120 min. after the insulin injection. At these times any symptoms or signs of hypoglycaemia are also noted, and the blood-pressure may be taken. It is of practical importance to notice the time of onset and termination of definite symptoms, and the more obvious symptoms, such as palpitations, headache and dizziness, faintness, sensations of warmth, and sweating, can be delineated with reasonable accuracy in onset and termination, though the tiredness and sleepiness which follow are usually more difficult to delimit. It is, however, the duration of the former more obvious symptoms that is noted. As mentioned below, undue severity or prolongation of the hypoglycaemia will usually be reflected in the symptoms; further, adequate warning of any need to interrupt the test will be given either by the prolongation of definite symptoms after 60 min., or by the appearance of symptoms of special significance which will be mentioned later under the heading of 'precautions'. After the last sample is taken at 120 min., 0.01 c.c. of 1:1000 adrenalin solution per kg. of body-weight is injected intramuscularly, and further capillary blood-sugar samples are taken 45 and 60 min. later.

*Modification of the insulin-adrenalin test for patients expected to be insulin-hypersensitive, that is, those suspected of hypopituitarism.* With patients suspected of panhypopituitarism of much degree, the dose of insulin is reduced to one-third the standard dose (0.033 units per kg.), but the test is otherwise unaltered. Since any clinically significant panpituitary defect probably increases insulin sensitivity at least threefold, the initial blood-sugar fall should still be maximal (that is, at least 45 per cent. of the fasting level). When this is not so, the test should be repeated with the standard dose. This modification reduces discomforts for the patient and makes the hypoglycaemic stress more comparable with the normal, so that the later portion

of the curve is a better index of the recovery mechanisms. It also enables the test to demonstrate the existence of insulin-hypersensitivity in these cases, since a maximal fall of blood-sugar is not produced normally with this dose. A comparable modification need not be worked out for insulin resistant states, since this test is not indicated in these conditions, but to complete our study of the test we have made a few tests in such conditions with larger doses of insulin.

*Precautions to be observed during and after the test.* If reasonable attention is paid to the symptoms and clinical state of the patient as each blood-sample is taken, no untoward effect should follow the test. Coma, of course, should never be allowed to occur, especially if any pituitary or adrenal defect is suspected. The most important danger sign to look for during the test is obvious clouding of consciousness; this will be evidenced by confusion in comprehending or answering questions. Slight defects in attention or mental acuity are not significant, but if there is any question of defect in the clarity of consciousness, the test should be immediately interrupted by adrenalin intramuscularly, or, if this is not effective within 10 min., by giving a drink containing sugar. This rule is probably adequate to cover all possible dangers, but the blood-pressure is an additional check. It should not be allowed to fall below 80 mm. systolic, unless it had been initially low, and then other signs of shock will herald any danger. Tests which have had to be stopped in this manner indicate sufficient susceptibility to hypoglycaemia for further samples to be unnecessary. In all cases where definite symptoms persist after the 60 min. sample, a close watch should be kept for the development of any of the above danger signs. As soon as the test is completed a good meal should be given, and then no further precautions are required, except that the patient should be advised not to leave an interval longer than three hours between meals for the rest of the day.

## APPENDIX II

### *Case Reports of Panhypopituitary Patients*

*Case 1.* H. S., a woman, aged 30 years in 1939. Diagnosis—panhypopituitarism, probably due to thrombosis after severe puerperal haemorrhage, associated complete myxoedema, and an Addisonian-like crisis after thyroid medication.

This patient was sent to hospital in 1939 as a case of myxoedema, complaining of dyspnoea with swelling of the ankles of one month's duration. There were no abnormalities in the family history, and up till seven years previously she had enjoyed good health. Since a childbirth in January 1932 she had never been well; a normal delivery had been followed by severe post partum haemorrhage associated with inversion of the uterus, which had necessitated a laparotomy and two blood transfusions. Even after this the puerperium had been eventful—she had had retention of urine, acute cystitis and acute pansinusitis, and had been febrile for some weeks. Since discharge from hospital she had had complete amenorrhoea, a constant tired feeling, dyspnoea on exertion, intermittent puffiness of the eyes and ankles, headaches, and occasional attacks of tightness round the heart. Her husband had noted that 'she had been almost constantly depressed, tired and listless, and had lost all libido; her memory had become increasingly poor, her speech and actions almost stupidly slow'. She had constant catarrh, two or three

colds each winter, and several attacks of cystitis. She was noticeably pale, had a few faint or dizzy turns each month, and had become sensitive to cold.

On examination she was of average build and nutrition, but looked slightly oedematous, specially in the face and ankles. Her weight was within a few pounds of her normal. Her face looked haggard, her skin was dry, atrophic and inelastic, and her colour pale and sallow (Plate 16, Fig. 2). The hair on her scalp and eyebrows was dry and fragile, and there was no hair in her axillae, on the pubis, or on the body generally. There were no significant findings in the examination of the chest, abdomen, or nervous system. Her blood-pressure was 90/72. Her teeth were moderately carious and there was mild gingivitis. Her breasts were atrophic with little sign of glandular tissue, her external genitalia atrophic, the vaginal mucous membrane pale and atrophic, and the internal genitalia infantile in size. Her blood count was red cells 4,200,000 per c.mm., haemoglobin 55 per cent., white cells 8,000 per c.mm. of which 63 per cent. were polymorphs. Her anaemia was not improved by iron or liver therapy. An X-ray of the skull revealed no abnormalities. Her serum total cholesterol was 120 mg. per 100 c.c., serum non-protein nitrogen 27 mg. per 100 c.c., and plasma proteins 4.8 and 6.6 gm. per 100 c.c. Her basal metabolic rate ranged between -35 and -45 per cent. Two insulin tolerance tests showed well-marked hypoglycaemia unresponsiveness (Table II), her urinary follicle stimulating hormone test on morning urine was negative for 5 m.u. per 100 c.c., and 17-ketosteroid assays were zero per 24 hours on six occasions. During her stay in hospital she was given a small dose of thyroid extract; on the third day she did not feel so well, and six days later began to become drowsy, confused, and weak. Her basal metabolic rate on the day previous to collapsing was -18 per cent.; after this collapse her condition resembled that of an Addisonian crisis, and so she was given large doses of intravenous saline. Three days later her condition had returned to normal. At the time of her collapse her blood-sugar had been 18 mg. per 100 c.c. (capillary) and 42 mg. per 100 c.c. (venous), and her total serum base 134 m. eq. per l.

*Case 2.* N. W., a woman aged 54 years in 1939. Diagnosis—panhypopituitarism, probably dependent on thrombosis during post partum haemorrhage; associated complete myxoedema.

This patient was sent to hospital in 1939 for treatment as a case of myxoedema, with complaints of increasing tiredness, anorexia, depression, and impairment of memory, associated with amenorrhoea of 17 years duration. Her family history was normal, and she was reputed to have had good health until a childbirth 17 years previously. This had been a difficult birth followed by profuse haemorrhage, and for the three days following she had been very exhausted and shocked. She never seemed to have regained her strength since then, but had been tired and sleepy, with little interest in life, had had intermittent swelling of her face and legs, poor appetite, and marked constipation. She had felt very 'slowed-up' mentally and physically, and had noticed impairment of her memory. She had become very sensitive to cold. Since then her condition had remained stationary till the previous 5 to 10 years in which it had gradually deteriorated in all these respects. About nine years previously she was reported to have had a slight stroke followed by a temporary facial paresis.

On examination she looked a haggard and aged woman, of poor nutrition, but not wasted (Plate 16, Fig. 3). Her face showed striking sallow pallor with general puffiness, especially round the eyelids. Her skin was dry,



atrophic, and inelastic with slight subcutaneous oedema. Her hair was dry, coarse, and fragile on the scalp, scanty on the eyebrows, and almost absent from the axillae, pubis, and body generally. Her extremities were strikingly cold, and her blood-pressure 95/60. Examination of the chest, abdomen, and nervous system showed no abnormality. She was normally orientated, but had considerable impairment of attention and was slow in action and thought. Her speech was a little thick. Her breasts were atrophic, as were also her external genitalia, vaginal mucosa, and uterus. No thyroid tissue was palpable. The mucous membrane at the edge of her tongue was atrophic. Her blood count was red cells 3,400,000 per c.mm., haemoglobin 75 per cent., white cells 5,000 per c.mm. The serum non-protein nitrogen was 20 mg. per 100 c.c., plasma-protein 7.4 gm. per 100 c.c., and total serum cholesterol 250 mg. per 100 c.c. Her heart was not enlarged by X-ray, and the electrocardiogram showed low voltage curves consistent with myxoedema. A skull X-ray showed no abnormalities. Several basal metabolic rate estimations ranged between -38 and -43 per cent. An insulin-adrenalin test showed hypoglycaemia unresponsiveness (Table II) and urinary hormone assays showed—follicle stimulating hormone test negative for 10 m. u. per 100 c.c. in morning urine, and zero 17-ketosteroid assays on three 24-hour samples of urine.

*Case 3.* M. W., a woman aged 34 years in 1938. Diagnosis—panhypopituitarism, probably due to thrombosis after post partum haemorrhage.

This patient was first seen at hospital in 1938 with the complaint of amenorrhoea following the birth of a child nine years previously. Before this she had always enjoyed good health and the family history was normal. The childbirth was followed by severe post partum haemorrhage for which she required transfusion, and on her return home she had had to remain in bed for a further three weeks. She was never able to nurse her child and did not have any swelling of her breasts. Since then she had been treated off and on for anaemia, and though she did not feel that she yet had her original vigour, she was able to manage her normal work and activities. She had had complete amenorrhoea since the childbirth, previously having had normal and regular periods since the age of 15 years.

On examination she was a woman of average build, good muscular development, and normal nutrition. Her weight was 8 st. 10½ lb., and height 5 ft. 1½ in. Her skin was thin and slightly dry, but moderately elastic; her colour was a pallid yellow. Examination of the chest, abdomen, and nervous system revealed no abnormalities. Scalp and eyebrow hair appeared normal, but axillary and pubic hair were absent. Her breasts were flat and there was no glandular tissue to be felt; her external genitalia looked small, the vaginal mucous membrane was pale and atrophic, and the uterus felt small. No endometrium could be obtained by the biopsy curette.

Estimations on fasting blood showed her serum cholesterol to be 367 mg. per 100 c.c., chlorides 109 m. eq. per l., and sodium 144.8 m. eq. per l. An X-ray of her skull was normal. Her basal metabolic rate was -23 per cent. Urinary hormone assays showed—follicle stimulating hormone test negative for 10 m. u. per 100 c.c. in morning urine, and 17-ketosteroid assay zero per 24 hours on three samples. The insulin-adrenalin test showed hypoglycaemia unresponsiveness (Table II).

*Case 4.* G. B., a woman aged 30 years in 1939. Diagnosis—panhypopituitarism of mild degree, probably due to thrombosis after post partum haemorrhage, or possibly to syphilitic invasion of the pituitary.

She first attended hospital in 1939 complaining of nervousness, weakness, and amenorrhoea of five years duration. Before 1934 she had been in very good health, except for hay-fever and asthma for seven years, and suspected contact with venereal disease at the age of 17 years. After the delivery of twins in that year she lost a considerable amount of blood. Since then she had not menstruated, had felt weak and tired, and had tended to cry easily.

On examination, her skin was pale and dry, there had been some loss of axillary hair, and of head and pubic hair. Her vaginal mucous membrane was pale and atrophic, and the uterus small. She looked well nourished, but had lost 17 lb. in weight during the illness. Two basal metabolic rate estimations were  $-4$  and  $-9$  per cent. The serum cholesterol was 117 mg. per 100 c.c. and serum-sodium 143.6 m. eq. per l. She was found to have a positive Hinton test in the blood, but her cerebrospinal fluid was normal and had a negative Wassermann reaction; luetic treatment was instituted. Her insulin tolerance (Table II) and 17-ketosteroid assay tests were both abnormal, the latter being 1.3 and 1.7 mg. in 24 hours. This case looked clinically the mildest of the cases studied.

*Case 5.* F. F., a woman aged 33 years in 1938. Diagnosis—chromophobe tumour producing panhypopituitarism; relieved considerably by X-ray therapy, and later complicated by pancreatic diabetes precipitated by an infection; subsequent partial relief of the panhypopituitarism by further X-ray therapy, thus making the pancreatic diabetes more evident. The features of her complex disorder of carbohydrate metabolism were revealed by tests.

This patient was first seen in 1933 with the complaints of increasing lack of energy, headaches with vomiting for six years associated with a loss of weight in the final three years, and amenorrhoea of one year's duration. Her family history had been normal, except that her mother suffered from diabetes which had commenced suddenly in 1930. The patient had three healthy siblings. She had enjoyed good health till the age of 10 years. At that time she had several illnesses during six months, comprising appendicitis and peritonitis, diphtheria, and a broken arm. Ever since then she had suffered from frequent colds, but otherwise had enjoyed normal health till the present illness. Previous to her present illness she had been 'a lot of fun and always the life of the party'. The illness began gradually in 1927 when she began to notice a 'lack of pep' and was readily tired. At the same time she began to get headaches often associated with vomiting, and her previously regular menstruation became infrequent, irregular, and less in amount. About this time her hair began to go grey, and she began to suffer from an irregular dull pain in her left iliac fossa, worse during her periods. In 1930 she had a laparotomy, and the left ovary, found to be full of cysts, was removed with relief of this pain, but her other symptoms continued to increase gradually. She was in bed nine weeks recovering from the operation, and she began to be sensitive to cold. By 1931 she was still just able to do her household tasks, but had very cold extremities, and felt tired and drowsy, but was unable to sleep when she went to bed. When examined in 1930 she had been found to have a dry skin and hair, and a systolic blood-pressure of 85/80. At this time she was given thyroid and ovarian tablets without relief. She had subsequently had several periods of thyroid treatment, also without relief. During 1932 she lost 47 lb. in weight, but since then her weight has been approximately constant. During 1933 she lost nearly all appetite, became

depressed, irritable, and hopeless of finding relief from her symptoms. Since 1933 she had had complete amenorrhoea. Her basal metabolic rate was first done in 1932 and found to be  $-29$  per cent.

On admission to hospital in 1933 X-rays revealed an enlarged sella turcica. She was first given a course of thyrotrophic hormone injections which raised her basal metabolic rate to  $+4$  per cent., but relieved no symptoms except her sensitivity to cold. She was then given a course of X-ray therapy, which relieved practically all her symptoms, though it did not restore her energy quite to its original level. Till 1938 she was able to do all her housework, and felt reasonably well. She had a course of X-ray therapy in 1935 which arrested a short recurrence of her previous symptoms. Two measurements of her basal metabolic rate in 1934 were  $-12$  per cent. and  $-19$  per cent. In August 1938 she developed a dental abscess, and was febrile for 10 days till an extraction was done; during this period she lost 12 lb. in weight and she never recovered her strength until after her subsequent admission to hospital in September. During the two months previous to this admission she developed polydipsia, polyuria, and attacks of cramp in her legs, associated with numbness and tingling of her toes. The week before her admission this culminated in what her doctor described as diabetic coma, at which time glycosuria was discovered. After this she was given insulin in the morning, 5 to 10 units depending on the results of the morning urine sugar test. On the fourth day of this treatment she had insulin hypoglycaemia, from which she was revived by the usual means, but still vomited for some hours; blurred vision, headaches, and dizziness then persisted for one to two weeks. Previous to this febrile episode no glycosuria had been found despite bi-monthly testing. It persisted, however, until she was put on a regulated diet of C170, P89, F134, after admission to hospital.

On examination in 1939, she was a woman of average build, musculature, and nutrition, looking considerably older than her years (Plate 16, Fig. 4). Her weight was 10 st. 5 lb. and height 5 ft. 5½ in. Her jaw was rather prominent, but there was no spacing of her teeth, and her extremities were normal in size. Her skin was dry, smooth, and rather inelastic, but not atrophic, and of a pale yellowish tint. Her extremities were cold even in bed, and her blood-pressure 80/60. Examination of the chest, abdomen, and nervous system revealed no abnormalities. Her visual fields and optic fundi were normal. Her external genitalia and uterus were small, and the vaginal mucous membrane atrophic. An X-ray of her skull showed further enlargement of the sella turcica. Her blood count was red cells 4,500,000 per c.mm., haemoglobin 85 per cent., white cells 10,000 per c.mm., of which 62 per cent. were polymorphs. Her blood non-protein nitrogen was 25 mg. per 100 c.c., serum alkali reserve 58.4 vols. per 100 c.c., chlorides 105 m. eq. per l., serum-calcium 8.9 mg. per 100 c.c., plasma-phosphates 3.7 mg. per 100 c.c., total cholesterol 284 mg. per 100 c.c. Her basal metabolic rate was  $-37$  and  $-31$  per cent. Her urinary hormone assays showed—negative follicle stimulating hormone test for 2.5 m.u. per 100 c.c. in morning urine, zero 17-ketosteroid assay on five occasions, and negative oestrin assays per 24 hours. Her carbohydrate metabolism was studied with several tests. Her urinary sugar disappeared after she was put on a diet of restricted carbohydrate; before that there had been 3.5 gm. in one 24-hour sample. The urinary tests done during her glucose tolerance test showed that her urinary threshold for sugar was normal. Three tolerance tests were done—plain glucose tolerance test, combined glucose and insulin tolerance test, and an insulin-adrenalin test. These showed a normal fasting level, a diabetic glucose tolerance test, increased

insulin sensitivity in the combined test, and hypoglycaemia unresponsiveness in the insulin-adrenalin test. A separate intravenous insulin tolerance test using one-third of the standard dose gave a maximal fall of blood-sugar with severe hypoglycaemic symptoms, giving further proof of the insulin hypersensitivity. There was relatively little capillary-venous difference in the combined glucose and insulin tolerance test.

She was then given a further course of X-ray therapy, which again relieved practically all her symptoms. She was re-admitted to hospital for a repetition of the tests four months later (March, 1939). Her general appearance was much better and she looked livelier, though most of the clinical features mentioned were still present. Her blood-pressure was unaltered. Her 17-ketosteroid assays were still zero, but striking differences were seen in her carbohydrate tests. Her fasting level of blood-sugar was now 260 mg. per 100 c.c. and she regularly showed morning glycosuria. In view of the high fasting level the plain glucose tolerance test was not repeated. The combined glucose and insulin test still showed insulin hypersensitivity, and an insulin-adrenalin test (after a preliminary reduction of the fasting level with insulin) still showed hypoglycaemia unresponsiveness, but not of quite so severe a degree. Although this latter test was not strictly comparable because of the preliminary insulin, the result was probably so abnormal as to be significant of defective responsiveness to hypoglycaemia. She now adheres to her measured diet, takes no insulin, and is reasonably well.

*Case 6.* T. V. O'R., a single man, aged 19 years in 1939. Diagnosis—residual panhypopituitarism after the pressure effects of a suprasellar cyst.

This patient was first seen at hospital in September 1931. He was the youngest of four healthy siblings, and his family history was normal. His previous development and general health had been normal although he had always been slim; he had had an appendicectomy in 1929. He complained of headache of one year's duration, and during the last few months of this period he had noticed failing vision, polyuria, and polydipsia. Even at this time he was described as having infantile features ('looked only eight years old'), a pallid waxy skin, and very scanty hair development, there being only a few pubic hairs. There was evidence of diabetes insipidus, his average daily intake of water being in the region of 70 oz., and the highest specific gravity found during a urine concentration test being 1013. An X-ray showed calcification above an enlarged sella turcica, destruction of its floor, and of the posterior clinoids. Examination of his vision showed blindness of the right eye and 20/70 vision in the left eye, where there was also temporal hemianopia. Craniotomy was performed in October 1931, and a small cyst, found anterior to the chiasma between the optic nerves, was curetted. Since then, seen at intervals in the clinic, he has remained infantile, dwarfed, and lacking in energy.

Since the operation growth has still occurred, but has been below normal, as shown by the following table:

Date	Height	Weight	Span	B.M.R. %
December 1931	4 ft. 2 in.	3 st. 10 lb.		—11 and —15
August 1932	4 ft. 3½ in.	3 st. 10 lb.		—18 and —18
June 1933	4 ft. 4 in.	4 st. 2 lb.		
July 1934	4 ft. 5 in.	4 st. 4 lb.		
December 1934	4 ft. 6 in.			—15 and —17
October 1937	4 ft. 8 in.		4 ft. 6 in.	—30 and —30
January 1938	4 ft. 8½ in.			—34
November 1938	4 ft. 8½ in.	5 st. 10 lb.		

During the first few years after the operation there were occasional attacks of nausea or vomiting during the night or in the morning, and one of these culminated in a convulsion. These were evidently hypoglycaemic attacks, and they have been rarer since 1937, when his appetite has been good and he has eaten better. He was admitted in 1937 for more detailed study, and again in 1939, when his clinical condition did not appear to have altered, and the results of these examinations may be taken together.

His general appearance, face, and build were those of a rather feminine, haggard, poorly nourished child (Plate 16, Fig. 5). In 1937 his measurements were: height 4 ft. 7 $\frac{3}{4}$  in., weight 4 st. 12 lb., span 4 ft. 6 in. His shoes were size 2 $\frac{1}{2}$ . His skin was pale, slightly yellow, dry and thin, with a few wrinkles; the subcutaneous tissue was very thin. Thin and dry hair was present on his head and eyebrows, but was almost completely absent elsewhere; there was no axillary, pubic, or facial hair. Examination of the chest and abdomen was negative and his blood-pressure 70/40. Vision was absent from the right eye and was still 20/70 in the left eye, but there was no longer any temporal hemianopia; bilateral optic atrophy was present. His genitalia were infantile, and the right testis was just at the external inguinal ring. X-rays of his hands, elbows, and pelvis showed many un-united epiphyses, the appearances being comparable to those seen in a child under 12 years of age. Lumbar puncture revealed normal cerebrospinal fluid, with 25 mg. of protein per 100 c.c., and normal pressure. Blood count, red cells 4,500,000 per c.mm. and haemoglobin 76 per cent., serum total cholesterol 250 mg. per 100 c.c., serum-calcium 10.4 per 100 c.c., plasma-phosphorus 4.7 mg. per 100 c.c., plasma-phosphatase 5 Bodansky units per 100 c.c. Basal metabolic rate -30 per cent. (two readings in 1937). A glucose tolerance test in 1937 gave the following figures—82 (fasting level), 120 ( $\frac{1}{2}$  hr.), 190 (1 hr.), 175 (2 hr.), 116 (4 hr.) mg. per 100 c.c. As shown in Table II, the intravenous insulin-adrenalin test done in 1939 showed hypoglycaemia unresponsiveness. Urinary hormone assays showed negative follicle stimulating hormone tests for 10 units in 1937 and 1939, and several negative 17-ketosteroid assays in 1939. During 1939 he was given a period of anterior pituitary therapy, during which his 17-ketosteroid assay became positive, but treatment was not continued long enough for clinical effects to be produced (Table I).

*Case 7.* G. B., a woman aged 45 years in 1939. Diagnosis—panhypopituitarism of unknown aetiology.

This patient attended hospital for asthma and hay-fever, and was referred to the clinic in 1939 because of the signs of endocrine disease. Her family history revealed no abnormalities. During adolescence she had suffered from many severe colds and began to get asthma from which she had never been free since. On inquiry she revealed that she had been undersized even in early childhood, but remarked that she was sure that she had grown during the previous few years. No sign of puberty had ever appeared. In July 1939 she injured her right wrist and on X-ray examination it was found that she had separated the distal radial epiphysis. Her only other previous illnesses were measles, chickenpox, severe pneumonia at the age of 12 years, and whooping-cough at the age of 13 years, at which time she was unconscious during a fit of coughing. However, she considered that apart from asthma her general health had been good until she began to suffer from hay-fever in 1933. She still worked efficiently as a housemaid, but since the hay-fever she had noticed that she tired more readily; when tired she had

suffered with backache in recent years. She had never at any period lost any significant amount of weight. During the last few years she had noticed dryness of her skin.

On examination she was a small, poorly developed, and thinly nourished woman looking somewhat older than her years (Plate 17, Fig. 6). Her height was 4 ft. 7 in. and weight 5 st. 10 lb.; she took size 6 in shoes and 6½ in gloves, and had thin slender fingers. Her skin was of a delicate texture, dry and somewhat atrophic; her complexion was pale, sallow, and yellow. The hair on her scalp and eyebrows was thin and dry, and there was practically none in her axillae, on the pubis, or on her body generally. Examination of the chest, abdomen, and nervous system revealed no abnormalities except the signs of asthma. Her blood-pressure was 120/90. Her visual fields and optic fundi were normal. There was practically no sign of breasts, and no glandular tissue was palpable. The external genitalia were also rudimentary, clitoris and labia being scarcely discernible, the vagina correspondingly atrophic, and only an extremely atrophic uterus palpable *per rectum*. An X-ray of her skull was normal; X-rays of her pelvis, knee, and hands showed diffuse osteoporosis, especially in the pelvic region, and several un-united epiphyses, the appearances being comparable to those seen in a girl of under 15 years. Her basal metabolic rate was +0.5 per cent. and -6 per cent., but this might have been an unduly high reading in view of her asthma. Her blood count was red cells 5,200,000 per c.mm., and haemoglobin 97 per cent. The serum-cholesterol was 260 mg. per 100 c.c.; plasma-protein 6.6 gm. per 100 c.c.; serum-calcium 10.4 mg. per 100 c.c.; plasma-phosphorus 4.0 mg. per 100 c.c., and plasma-phosphatase 3.4 Bodansky units per 100 c.c. An insulin-adrenalin test showed hypoglycaemia unresponsiveness (Table II). Urinary hormone assays showed a negative follicle stimulating hormone test for 10 m.u. per 100 c.c. in morning urine, and zero 17-ketosteroid assay on three 24-hour samples.

*Case 8. D. A., a youth aged 18 years in 1939. Diagnosis—dwarfism due to panhypopituitarism.*

This patient was first seen in 1934 on account of retarded growth. His family were all healthy and tall, and the only sibling, aged 10 years, was taller than the patient. His health was quite normal till about the age of five years when his rate of growth began to diminish. Previously there had been no ill-health, but latterly his appetite and energy had been deficient, and he had been very sensitive to cold. There had been no sign of puberty. When seen in 1934 little abnormal was noticed beyond the lack of growth, which was found to be associated with epiphyseal development corresponding to that normally present at eight years, and a basal metabolic rate of -13 per cent. After an initial period of anterior pituitary extract injections which did not alter his growth, he was treated with thyroid, which has been continued ever since with occasional changes in dosage. There were no measurements previous to treatment available for comparison, but he has gained an average of 1 in. in height and 4½ lb. in weight annually during these years of treatment. Further X-rays in 1939 showed one new ossification centre in the wrist, the appearances corresponding with the normal for 9 to 10 years. He has progressed well at school.

On examination in 1939 his general appearance was that of a rather delicate boy of good nutrition, but poor muscular development. His capacity for exercise had been noticed to be poor. His skin was normally coloured and moist, but rather thin and delicate. His face was not at all wrinkled,

but did not have much vivacity (Plate 17, Fig. 7). The hair on his head and eyebrows was normal, but a little dry; there was no axillary, pubic, or facial hair, and body hair was very slight. Examination of the chest, abdomen, and nervous system revealed no abnormalities. His blood-pressure was 90/60. The blood count was red cells 3,800,000 per c.mm., and haemoglobin 59 per cent. The anaemia was not improved by treatment with liver or iron. His genitalia were normal for pre-puberty. The serum-cholesterol was 219 mg. per 100 c.c. His basal metabolic rate when not under treatment with thyroid was -20 per cent. Insulin tolerance tests (Table II) revealed hypoglycaemia unresponsiveness. Urinary hormone assays showed a negative follicle stimulating hormone test for 10 m.u. per 100 c.c. of morning urine, and five negative 17-ketosteroid assays per 24 hours. During 1939, from July 10 to September 27, he was given a series of injections of 'doca' (desoxycorticosterone), starting at 5 mg. daily and later increased to 10 mg. daily. The first few injections upset him, producing a headache, but this did not recur; there was no improvement in his sense of well-being, or any change in the rate of growth.

*Case 9. J. C., a woman aged 20 years in 1939. Diagnosis—dwarfism due to panhypopituitarism; aetiology possibly related to asphyxia neonatorum.*

This patient was admitted to hospital with the complaint of dwarfism. She was the eldest of six male and two female siblings, and her height was between that of her sisters aged 8 and 10 years; her parents were healthy Italians of heights 5 ft. 10 in. and 5 ft. 4 in. She was a full term child of normal weight, who when born was 'blue' and 'almost given up for dead'. She gained weight normally during the first years and passed her milestones of talking and first teething normally. She suffered from a congenital dislocation of the left hip, for which she was treated in the Children's Hospital, Boston. During the first year she had convulsions, which have not recurred, and measles. As a child she had suffered from nocturnal enuresis, which still occurred occasionally. Her parents stated that her growth had been delayed since the age of three years, and that there had been practically none since the 13th year; her school measurements, from 1926 onwards, were obtained and indicated that a slow growth was still proceeding; an average gain between 1926 and 1939 of about  $\frac{3}{4}$  in. and just over 2 lb. per annum having been recorded. There had been no sign of puberty. Even as a child she had a poor appetite, and, especially during later years, she had noticed easy fatigability and sensitivity to cold.

On examination in 1939 her build was that of a thin delicate child, but her face had a striking combination of infantile and aged features (Plate 17, Fig. 8). Her measurements were standing height 3 ft. 10½ in., sitting height 24 in., weight 3 st. 10 lb. Her skin was thin, dry, and of a sallow yellow colour. There was no axillary or pubic hair and scanty body hair; the hair on the head and eyebrows was thin and dry. Her hands were delicate and slender. Physical examination of her chest, abdomen, and nervous system revealed no abnormalities. Her blood-pressure was 84/60. The electrocardiogram was normal. There were no secondary sexual characters, and her genitalia were those of a young child.

X-ray of the skull showed a normal sella turcica. X-rays of her epiphyses showed many un-united epiphyses, the appearances corresponding with those of a normal child of under 12 years. Her dental development was estimated by Dr. Strock to correspond with the normal for 10 years. Her blood count was red cells 4,000,000 per c.mm., haemoglobin 51 per cent., white cells 8,300

per c.mm., of which 78 per cent were polymorphs. The serum-calcium was 10.0 mg. per 100 c.c., plasma-phosphate 4.7 mg. per 100 c.c., plasma-phosphatase 5.3 Bodansky units per 100 c.c., plasma-protein 6.6 gm. per 100 c.c., serum total cholesterol 146 mg. per 100 c.c., sodium 122.9 m.eq. per l., potassium 4.66 m. eq. per l., chlorides 531 mg. per 100 c.c. Her basal metabolic rate was -32 per cent. relative to height and -33 per cent. relative to weight. Insulin tolerance tests (Table II) revealed hypoglycaemia unresponsiveness. Urinary hormone assays showed a negative follicle stimulating hormone test for 5 m.u. per 100 c.c. of morning urine and four negative 17-ketosteroid assays per 24 hours. She was given thyroid extract gr. 1 per diem. From August 1 to September 22 she was also given injections of 'doca' (desoxycorticosterone), 10 mg. per diem for some of the period. This did not improve her energy or health in any way, and there was no noticeable change in her rate of growth. It is too early yet to assess the effectiveness of the thyroid treatment.

*Case 10.* G. K., a single man aged 55 years in 1939. Diagnosis—panhypopituitarism of unknown aetiology.

He first came to hospital in 1928 complaining of weakness, poor appetite, and loss of hair since 1922. The patient was a Greek who had come to America shortly after the onset of his illness; he had four apparently healthy siblings, but no further details were available about his family. Previous to this illness he had always been strong and healthy; the only illness that he remembered was an attack of malaria between 1900 and 1905. His symptoms had begun after a short vague febrile illness. He first noticed a loss of his skin tan, followed by a disappearance of the hair from his body, and later from the pubes. At the same time he noticed a cessation of nocturnal emissions, he became impotent, and his appetite and strength began to go. Since about two years after the onset he had noticed that his legs had swelled in the evenings, he had had dyspnoea on exertion and suffered intermittently from attacks of diarrhoea. His clinical condition when first seen in 1927 was evidently very similar to that in 1939. Since 1928 he had had occasional dizzy spells, but had worked as a cook for the 18 years prior to admission to hospital.

He was well built and well nourished. His weight was 9 st. 4 lb. and height 5 ft. 7½ in. His face is well shown in Plate 17, Fig. 9. His skin was pale yellow, smooth, dry, atrophic, and inelastic; his face was slightly puffy under this thick skin. He had scanty pubic hair and eyebrows, but no hair in the axillae or on the beard area.

Physical examination of the heart and lungs revealed no abnormalities, but an X-ray showed evidence of calcified tuberculous lesions at the lung apices, and an electrocardiogram showed signs of myocardial deficiency. Examination of the abdomen was negative, and a barium meal and enema revealed no abnormalities. He had had a tendency to constipation since his illness. His blood-pressure was 135/80, and there were no indications of arterial disease. His penis was of normal size, his scrotal skin smooth and thin, and his testes normal in size though possibly a little soft; *per rectum* there was scarcely any prostatic tissue to be felt.

A gastric test meal in 1928 revealed achlorhydria. A blood count in 1939 was red cells 4,180,000 per c.mm., haemoglobin 78 per cent., white cells 7,000 per c.mm. His serum-calcium was 10.2 mg. per 100 c.c., plasma-phosphate 3.3 mg. per 100 c.c., and phosphatase 3.1 Bodansky units per 100 c.c., plasma-cholesterol 100 mg. per 100 c.c. His basal metabolic rate



was repeatedly estimated and found to vary from  $-27$  to  $-30$  per cent. independently of treatment. During a period of thyroid treatment in 1927 the basal metabolic rate was raised to  $-5$  per cent. without any relief of symptoms; the treatment was discontinued as it made him feel worse. He was treated in 1928 with a combination of thyroid and anterior pituitary extract without any benefit.

He returned to hospital in 1939 on account of an increase of his symptoms after a cold—increased swelling of the ankles, general coldness, and weakness. After admission he had an attack of vomiting and an Addisonian-like crisis developed; he rapidly became confused and stuporose, febrile, complaining of headache and abdominal pain, and showing moderate muscular rigidity. A sample of venous blood examined at this time showed plasma-chlorides 76 m. eq. per l., plasma-protein 7.0 gm. per 100 c.c., serum-sodium 123.5 m. eq. per l., and non-protein nitrogen 13 mg. per 100 c.c., sugar 92 mg. per 100 c.c. Intravenous saline medication rapidly allayed his severe symptoms, and after a few days the fever subsided and he returned to his usual clinical state.

Urinary hormone assays in 1939 showed a negative follicle stimulating hormone test for 10 m. u. per 100 c.c. of morning urine, and zero 17-ketosteroids per 24 hours on several assays. An insulin tolerance test after diet preparation showed marked hypoglycaemia unresponsiveness (Table II).

During 1939 he was given three courses of treatment. From November 27 he was given injections of chorionic gonadotrophin, 1,000 i. u. daily; there was no noticeable clinical effect during this short course, but it raised the 17-ketosteroid assay to positive figures. From July 7 to October 13 he was given testosterone injections, small doses being used at first, and from July 31st in a dosage of 25 mg. thrice weekly. During this treatment he noticed a slight improvement in his general strength, a recurrence of erections and some emissions, and his 17-ketosteroid assays became positive. From November 9 to 20 he was given injections of 'doca' (desoxycorticosterone) commencing at 5 mg. daily, and 30 mg. daily from November 15. From the fourth day he developed oedema, which subsided three days after cessation of treatment. During this treatment his general strength was less, and there was no evidence of any benefit.

### APPENDIX III

#### *Case Reports of Patients with Myxoedema and Anorexia Nervosa*

##### *Myxoedema.*

*Case 11.* H. C., a woman aged 63 years in 1939. There had been no significant previous illness. For the previous 10 years there had been fatiguability and weakness of gradual onset, associated with mental sluggishness and poor memory. Her hair had also gradually fallen out and become brittle, and she had also noticed dyspnoea on exertion and slight dyspepsia. On examination she weighed 13 st. 6 lb. and presented the classical picture of myxoedema—slow cerebration and general activity, thick tongue and lips, and thick dry skin. She also had essential hypertension. Her serum-cholesterol was 342 mg. per 100 c.c., and basal metabolic rate  $-26$  per cent. The results of the insulin tolerance and 17-ketosteroid assay tests are shown in Table III. She was subsequently discharged very much improved on thyroid treatment.

*Case 12.* M. B., a woman aged 72 years in 1940. A diagnosis of myxoedema had been established in this patient 18 years before admission, and she had

been put on thyroid therapy with complete relief of her symptoms. A year previously she had omitted her treatment because of financial difficulties, and four months later developed swelling of the face, dry skin, falling hair, gain in weight, with decreased perspiration, and decreased tolerance to cold. On examination she showed the classical picture of myxoedema—loss of the lateral half of the eyebrows, enlarged tongue, obesity (weight 11 st. 2 lb.), and a dry and scaly skin. Her serum-cholesterol was 362 mg. per 100 c.c., and the basal metabolic rate —50, —35, —42, —36 per cent. The results of the insulin tolerance and 17-ketosteroid assay tests are shown in Table III. She was again put on treatment with thyroid, and her basal metabolic rate rose to —12 per cent., with relief of her recently acquired symptoms.

*Case 13.* M. H., a woman aged 28 years in 1940. She was first seen in 1939 complaining of joint pains of seven years duration which had occurred since the birth of her first child. During this time she had also noticed mental and physical sluggishness. During the earlier part of her illness she had taken thyroid extract with much improvement, but had later stopped this treatment. She had had two other pregnancies in 1934 and 1935, after the last of which she began to gain weight and to suffer from narcolepsy-like symptoms. On examination in 1939, she was obese, her skin was dry though rather thin, and her hair was coarse, with normal pubic hair, and a small amount of axillary hair. Her serum-cholesterol was 312 mg. per 100 c.c., and basal metabolic rates were —25, —35, and —39 per cent. The results of the insulin tolerance and 17-ketosteroid assay tests are shown in Table III. She was given thyroid treatment, with relief of her symptoms and the return to normal of her scanty and irregular periods.

#### *Anorexia nervosa.*

*Case 21.* G. H., a woman aged 26 years in 1939. She was admitted because of loss of energy and appetite, together with vomiting, since a series of family troubles in 1932. Previously she had always been in good health, except for postural lordosis which had been treated on and off since the age of 14 years. Early in 1932 her father died, she lost her job, and then broke off her engagement. She gradually deteriorated, losing weight and appetite first; she would vomit if she ate much. Her periods became scanty and infrequent. During this time she lost 20 lb. in weight. On examination she showed moderate malnutrition. The results of her insulin and 17-ketosteroid assay tests are shown in Table III. With treatment she has gradually gained some of her lost weight and strength.

*Case 22.* M. E., a woman aged 19 years in 1937. She was admitted to hospital in 1937 because of refusal of food, loss of 42 lb. in weight in 18 months, latterly with complete amenorrhoea, depression, headaches, and fainting attacks. All the family were of poor physical constitution. She had previously had fair health, and had won medals at school for running. She had had chorea at the age of nine years, and been periodically ill with anaemia for short periods. Menses had begun at 12 years and been normal till her illness. She had mixed fairly well at school, but been rather quiet and readily upset. She had never had any special male friends. Since leaving school she had found her work heavy, and had had difficulties with friends, and began to have fatigue, headaches, and vomiting attacks. Just before the onset of these symptoms, she had begun to talk of an imaginary male friend. Her fatigue increased, amenorrhoea set in, and she lost 42 lb. in weight. She had been

admitted to another hospital and after five months was discharged with her physical condition restored, her weight and periods then being normal. Immediately on discharge she relapsed to her previous state and so was admitted to the Maudsley Hospital. On admission she was mostly depressed, cried frequently, and had few interests. She had no desire for food, was emaciated and listless, and her skin was pallid and a little dry, but not atrophic. Her scalp hair had been falling out, the pubic and axillary hair were normal, and there was a new growth of downy hair on her legs, arms, and face. Her blood-pressure was 90/60, and her extremities cold. Her basal metabolic rate was  $-25$  per cent. Her blood count was red cells 4,000,000 per c.mm., and haemoglobin 70 per cent. Her insulin tolerance test (Table III) was normal. After a prolonged period in hospital she finally recovered her physical health.

*Case 23.* M. P., a woman aged 31 years in 1936. This patient was first admitted to hospital in May 1936, complaining of amenorrhoea, loss of 35 lb. in weight, general weakness, indigestion and vomiting, and giddiness, of one year's duration. Her family history was normal. Till the present illness her health had been normal; her menses had begun at 17 years, with large but regular losses. She is described as having been a lively, healthy, 'good' girl. She had had a number of girl friends, but never any male friends; her mood had been steadily cheerful, and she had been mildly obsessional. The prodromata of her present illness had begun at the age of 19 years. The precipitating factors were not clear, but she had begun to have an aching pain in her right iliac fossa for which she had had a series of operations, including an appendicectomy, none having much effect till a cystic ovary was removed in 1934. During these seven years she also gradually lost strength and appetite, though continuing to work. After her last operation she suffered increase of all symptoms. Finally, about the middle of 1935 rapid deterioration commenced—in addition to the loss of strength and appetite, she developed vomiting, complete amenorrhoea, constipation, a rapid loss of 30 lb. in weight in a few months, headaches, and giddiness. Her condition on admission (Plate 18, Fig. 10) was one of considerable emaciation. She maintained a relatively cheerful, resigned attitude, except for occasional lapses of crying. She considered her illness physical. Her skin was thin, dry and atrophic; there was practically no axillary hair, the pubic hair was normal, her eyebrows and scalp hair were slightly reduced. There was a fine downy growth of new hair on her extremities and face. Her blood-pressure was 90/70, and her extremities cold. X-ray of skull was normal. The genitalia were atrophic. Blood count, red cells 4,500,000 per c.mm., haemoglobin 70 per cent. Several basal metabolic rate tests ranged from  $-9$  to  $-18$  per cent. Her insulin tolerance test (Table III) was normal. Unfortunately treatment did not alter her condition.

*Case 24.* M. P., a girl aged 17 years in 1936. This patient was first admitted to hospital in 1936, with a loss of 36 lb. in weight, anorexia, and complete amenorrhoea of 12 months duration. She was an only child, her father was neurotic, and her mother had died when she was three years old. She was brought up by people who did not understand her, and she was introspective, lonely, and bad tempered, though also very affectionate when circumstances permitted. At school she had been popular, always craving attention and sympathy; she was always active, obstinate, highly imaginative, and very intelligent. Her illness began in her last year at school. She had just been very depressed over losing a close friend, was intensely preoccupied with her

confirmation, and at the same time was being given many extra activities and responsibilities at school for all of which she was seeming to get little appreciation or sympathy. On her next holiday, as people had been teasing her for being fat she curtailed her eating. When she found she was losing weight, treatment and attention were directed to it, all of which seemed to enhance her avoidance of food into a compulsion against which she became increasingly powerless, though recognizing it as ridiculous, and often feeling hungry. From this point she became unable to resist the compulsion, depressed, and weak, though still persisting in an excessive activity 'as if there were something driving me to destroy me and I couldn't stop it'. She wished to die by the time she was admitted to hospital. On admission she was depressed, but talked with interest about her experiences and sensations; she could not be persuaded to eat and used many tricks to avoid taking food. She was emaciated (Plate 18, Fig. 11), and her skin was thin, dry, and atrophic. There was some axillary and pubic hair, but her scalp and eyebrow hair were normal. The genitalia were atrophic. Her blood-pressure was 80/60, and her extremities cold and blue. X-ray of the skull was normal. Basal metabolic rate tests ranged between  $-20$  and  $-25$  per cent. After a careful diet preparation for a week, her insulin tolerance test was normal (Table III). After prolonged resistance to treatment, she suddenly began to eat in November 1937, and by August 1938 she weighed 140 lb. (Plate 18, Fig. 12). Since May 1938 she has been having irregular periods. She took up nursing, and in 1941 her physical condition was normal, though she was still somewhat unstable emotionally.

*Case 25.* A. V., a girl aged 20 years in 1939. This patient came to hospital complaining of amenorrhoea of 18 months duration. Her previous health had been relatively normal. Two months before the onset of her illness her father had died, and she had been much upset. At the same time she went off her food and lost 30 lb. in weight. On admission she was found to be moderately emaciated, but had relatively normal hair distribution, including axillary and pubic hair; there was even a tendency towards hirsutism. Her genitalia were atrophic, but her skin did not look abnormal on physical examination. Her serum-cholesterol was 151 mg. per 100 c.c., basal metabolic rate  $-23$  per cent., urinary follicle stimulating hormone test negative. Her insulin tolerance test was nearly normal, and her urinary 17-ketosteroid assays 7.5 mg. and 8.9 mg. in 24 hours. During a month of diet and other treatment she regained 12 lb. in weight, and further progress seemed probable.

*Case 26.* M. C., a woman aged 27 years in 1938. (? Secondary slight panhypopituitarism.) This patient was admitted to hospital in 1938 complaining of fatigue for 10 years, and of amenorrhoea and indigestion for nine years. Her family history was normal and she had had no significant illnesses before the present one. She had always been a lively, irrepressible girl, given to tempers and jealousy, and she never 'got on' with her family. Her periods had started at the age of 12 years and been normal till the onset of this illness; since then after three months irregularity they had been absent. Her present illness had begun with fatigue and irritability at the age of 17 years after leaving school, an emotional upset associated with her failure in achieving many ambitions, and general difficulties in making friends. She would practice dancing incessantly despite increasing fatigue and irritability and family conflicts. From the onset her appetite flagged and indigestion

developed. At the age of 22 years, after an attack of influenza, she had alopecia areata which cleared up in five months. During the 10 years of her illness she had worked only irregularly, and had gradually lost more and more energy, appetite, and 27 lb. in weight during 1938. During the previous five years she had spent some days of each week in bed, and had been miserable, discouraged, and introspective most of the time. On admission to hospital in 1938 she was voluble about her complaints, and dramatized much during interviews. While reciting her complaints she appeared cheerful, and showed preoccupation with her physical disabilities and ambitious dreams. Her nutrition and physical development were poor, and she looked infantile. Her skin was slightly dry and pale, but not atrophic. Her eyebrows, scalp hair, and pubic hair were abundant, but the axillary hair small in amount. Her genitalia were atrophic. There were no other abnormalities on physical examination. X-rays of the long bones and skull were normal. Her serum-sodium was 148.1 m.eq. per l., chloride 106.7 m.eq. per l., and plasma-cholesterol 90 mg. per 100 c.c. Blood count, red cells 4,500,000 per c.mm., and haemoglobin 90 per cent. Four basal metabolic rate readings varied between -20 and -30 per cent. Wassermann reaction negative. Her urinary 17-ketosteroid assay was 2.7 mg. per 24 hours; the insulin tolerance test showed some hypoglycaemia unresponsiveness, but her diet preparation had probably been inadequate. Treatment proved difficult in view of the chronicity.

*Case 27.* H. L., a girl aged 15 years in 1939. She was admitted to hospital because of loss of weight and amenorrhoea of three years duration. Her previous health had been normal. Her periods had begun at the age of 11 years and been normally regular for at least a year. Towards the end of her 11th year, after the death of her father, she began to have spells of crying and sulking, and lost her appetite. After a year of irregular periods, complete amenorrhoea set in. During the period of this illness she lost 23 lb. in weight. On admission she was found to be emaciated, but with normal hair distribution, including axillary hair, and with normal skin texture. Her blood-pressure was 100/76, and her pulse 50 to 60. Her height was 5 ft. 4 in. and her basal metabolic rate -25 per cent. The urinary follicle stimulating hormone test was negative, and her blood count normal. Her insulin tolerance test was not normal, but her diet preparation for it had not been adequate; her urinary 17-ketosteroids were high normal (Table III). It was not possible to follow her subsequent progress.

*Case 28.* J. F., a woman aged 25 years. She was admitted to hospital in 1936 complaining of loss of 42 lb. in weight and disinclination to eat, with amenorrhoea of  $4\frac{1}{2}$  years duration. Her family history was normal. Her health, previous to marriage six years before, had been normal; she is described as having been energetic, meticulous, upset by small worries, and showing rapid changes of mood. Her marriage had never been satisfactory. After a year and a half, she began to lose her appetite and energy and developed complete amenorrhoea. She would vomit if she exerted herself or ate any quantity of food, and she had fainting turns. She became increasingly irritable and suffered from headaches. On admission she was seen to be grossly emaciated and had lost 39 lb. in weight. She was childishly irritable, and could not be persuaded to eat. Her blood-pressure was 95/55, her blood count normal, and her basal metabolic rate -24 per cent. X-ray of her skull was normal. She left hospital before treatment could be begun.

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FIG. 2. Case 1. H.S. at the age of 30 years



FIG. 3. Case 2. N.W. at the age of 54 years



FIG. 4. Case 5. F.F. at the age of 35 years

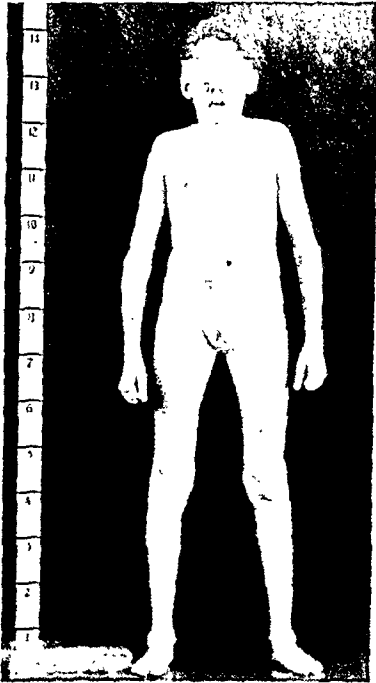


FIG. 5. Case 6. T.V.O.R. at the age of 18 years





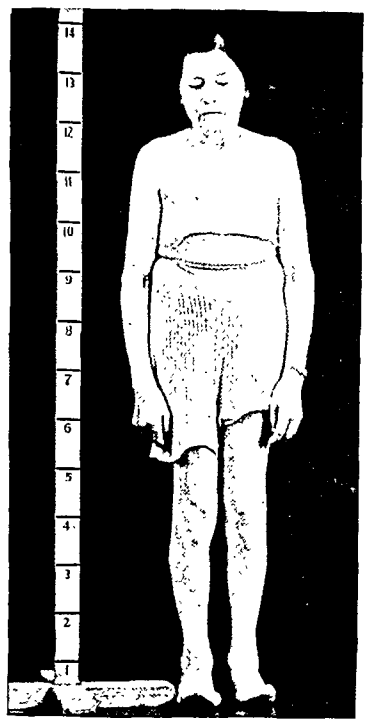


FIG. 6. Case 7. G.B. at the age of 45 years

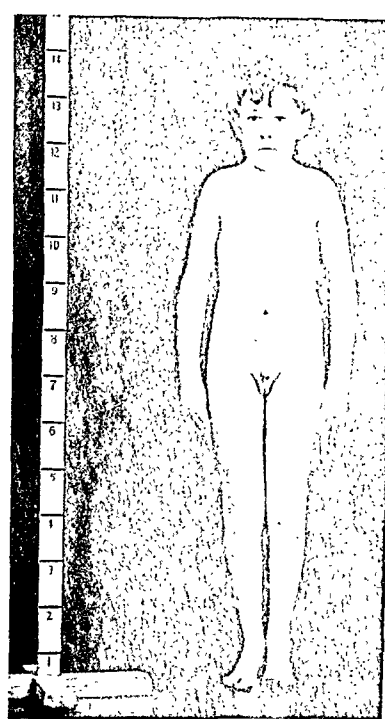


FIG. 7. Case 8. D.A. at the age of 19 years



FIG. 8. Case 9. J.C. at the age of 21 years

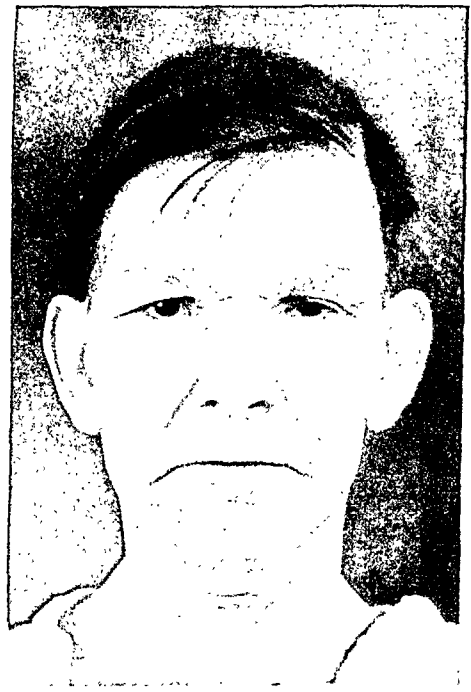


FIG. 9. Case 10. G.K. at the age of 53 years



# PROCEEDINGS OF THE ASSOCIATION OF PHYSICIANS OF GREAT BRITAIN AND IRELAND

1941

## THIRTY-FIFTH ANNUAL GENERAL MEETING

THE THIRTY-FIFTH ANNUAL GENERAL MEETING was held in Oxford at the School of Geography on Saturday, April 19, 1941. The attendance book was signed by 91 members. The proceedings began at 10 a.m.

*The President*, Sir Robert Hutchison, was in the Chair.

*The Minutes* of the last Annual General Meeting, having been published in the *Quarterly Journal of Medicine*, were taken as read and confirmed.

*The Treasurer* presented the Annual Accounts, which showed a balance of £849. From this the account from the Clarendon Press, which had not yet been received, must be deducted, leaving a balance of approximately £450.

*The Secretary* referred to the growth and size of the Association. There was too long a waiting list, and the admission of younger physicians might be speeded up by relaxing the conditions of Extra-Ordinary Membership.

### *Election of Officers*

*President.* Sir Farquhar Buzzard was elected President. On his election he took the Chair, and expressed the thanks of the Association to the retiring President, Sir Robert Hutchison.

Election of Officers, Executive Committee, Honorary Member, Extra-Ordinary Members, and Ordinary Members then followed.

### *Executive Committee*

*President.* Sir Farquhar Buzzard.

*Treasurer.* Col. H. Letheby Tidy.

*Secretary.* Professor L. J. Witts.

#### *Members for England:*

Dr. A. E. Barnes.  
Dr. J. Murray Bligh.  
Dr. George Graham.  
Dr. J. L. Livingstone.  
Dr. J. Parkinson.  
Professor L. G. Parsons.

#### *Members for Scotland:*

Professor R. S. Aitken.  
Professor A. W. Harrington.  
Professor D. Murray Lyon.

#### *Members for Ireland:*

Dr. F. M. B. Allen.  
Professor G. Bewley.  
Dr. J. A. Smyth.

#### *Honorary Member*

Sir Arthur F. Hurst.

*Extra-Ordinary Members*

Dr. C. W. Buckley.  
 Dr. J. Cowan.  
 Dr. W. Edgecombe.  
 Dr. Ivy Mackenzie.  
 Sir Edmund Spriggs.

*Ordinary Members*

Stanley Alstead, M.D., Assistant Physician, Stobhill Hospital, Glasgow.  
 Harold William Fullerton, M.D., Lecturer in Medicine, University of Aberdeen.  
 Frank Patrick Lee Lander, M.D., Assistant Physician, Royal Free Hospital.  
 David Michael Mitchell, M.D., Visiting Physician, Dr. Steevens' Hospital, Dublin.  
 George White Pickering, F.R.C.P., Professor of Medicine, St. Mary's Hospital.  
 Norman Swift Plummer, M.D., Assistant Physician, Charing Cross Hospital.  
 Ronald Bodley Scott, D.M., Assistant to Medical Unit, St. Bartholomew's Hospital.  
 Samuel Levy Simpson, M.D., Physician, Willesden General Hospital.

## SCIENTIFIC BUSINESS

*Saturday Morning*

1. DR. ROBERT COOPE gave a communication on *Pleural Blebs and Spontaneous Pneumothorax*, describing the clinical, radiological, and thorascopic appearances in a patient suffering from a 'pneumothorax of scarred lung'.

DR. F. G. CHANDLER showed a number of slides to illustrate that spontaneous pneumothorax might arise from acquired emphysematous bullae or from congenital cysts. In his experience congenital cystic disease of the lungs was the commonest cause of spontaneous pneumothorax. DR. PARKES WEBER pointed out that there was nothing new in the conception of non-tuberculous spontaneous pneumothorax. Twenty years ago he had collected references to 127 communications on this subject. The chief recent addition to knowledge was the frequency of congenital cysts.

2. DR. WILLIAM EVANS spoke on *A Comparison of the Mercurial Diuretics in the Treatment of Cardiac Oedema*. He gave the results of an investigation which tried to determine the best preparation to use, the best method of giving it, and the most efficient means of augmenting its diuretic effect. Diuresis from 307 separate injections was recorded, and 507 trials were devoted to premedication. When given intravenously neptal and esidrone induced greater diuresis than salyrgan and much greater diuresis than mersalyl. With only few exceptions intravenous injection induced a greater response than intramuscular injection. When the mercurial salts were given by mouth, neptal tablets gave far better results than mersalyl and salyrgan tablets, and novurit suppositories. When 13 different methods of premedication were compared, 30 gr. of ammonium chloride, given in four chocolate-coated enteric tablets two hours before the mercurial preparation, proved best in augmenting its natural diuretic effect.

3. DR. R. PLATT discussed the *Treatment of Nephrosis by Intravenous Plasma* and described a case in which after other treatment had failed the injection of concentrated human plasma was followed by diuresis with the loss of about 7 st. in weight within 2½ months. Other cases, mostly of subacute nephritis, had been treated in the same way, but without any beneficial effect. The literature of the subject was briefly reviewed.

The above two communications were discussed together. DR. D. A. K. BLACK reported two cases of hydraemic nephritis which had not benefited from concentrated serum. PROFESSOR A. W. M. ELLIS and DR. CLIFFORD WILSON reported four cases, only one of which had benefited, and in that case there was a rigor. PROFESSOR L. S. P. DAVIDSON had seen no beneficial effect, and found that the injected protein was rapidly excreted. PROFESSOR C. BRUCE PERRY had noted increased albuminuria and urticaria following the injection of concentrated serum. DR. TERENCE EAST confirmed the action of febrile infection as a diuretic factor in nephrosis. DR. GEORGE GRAHAM stressed the importance of securing a normal alkali reserve. DR. PLATT in reply rebutted any suggestion that the diuresis in his patient was due to infection or rigors. He had seen one sudden death after injection of neptal, and other patients had collapsed. DR. WILLIAM EVANS agreed that excessive fluid depletion was unwise in cases of oedema.

4. DRS. D. BEALE, T. H. BELT, and E. G. L. BYWATERS (introduced by DR. J. McMICHAEL) described *Renal Failure in Crushing Injuries*. Patients with 'traumatic oedema' after burial under debris show, after recovery from the initial loss of blood-volume, a rising blood-pressure and signs of renal failure. Pigmented casts (? haemoglobin or myohaemoglobin) appear in the often scanty urine, and the majority die within eight days with a rising blood-urea and serum-potassium. Autopsy shows muscle necrosis and severe renal damage, with pigmented casts. Apart from direct pressure ischaemia, secondary muscle necrosis from vascular damage may be associated with this picture, e. g. after car accidents. Recovery may occur, especially in crushes lasting longer than 15 hours, due perhaps to 'natural amputation'.

DR. A. H. T. ROBB-SMITH thought that it was desirable to study the nature of the pigments in the plasma and urine both in these patients and in cases of burns. PROFESSOR J. A. RYLE thought that we should pay close attention to renal function in all forms of trauma. PROFESSOR A. W. M. ELLIS discussed the effect of incision of the swollen limbs. DR. R. PLATT suggested that rapid anaemia might be a factor and DR. GEORGE GRAHAM pointed out that the alkali reserve might be abnormal. DR. BYWATERS replied to various points, and indicated that incision was not very effective.

5. MISS E. M. WIDDOWSON (introduced by PROFESSOR J. A. RYLE) described *An Experimental Study of Rationing*. A diet was devised which was biochemically satisfactory and which it was considered this country might aim at providing in a real food crisis. All foods were severely restricted except brown bread (reinforced with calcium carbonate), potatoes, and other vegetables. Five adults lived on this diet for 3½ months. They took several weeks to adapt themselves fully to the new regime, but they gradually ate more and more of the unrationed foods until their caloric intakes were as high as they were before the experiment began. About 1½ lb. of bread and 2 lb. of potatoes a day had to be eaten by some of the party to make up for the fats, sugar, and meat which were so strictly rationed. The diet contained generous amounts of protein, calcium, phosphorus and iron, and of vitamins B<sub>1</sub> and C. The subjects proved themselves to be in excellent physical condition at the end, and were able to carry out strenuous exercise consisting of long-distance cycling, walking, and mountain climbing.

6. DR. R. A. McCANCE discussed *Wheat in a War-time Dietary*. Calcium balance experiments each lasting three weeks were carried out on four healthy men and four women. Calcium was found to be absorbed less freely from brown than from white bread dietaries. The differences, which were considerable, were shown by all the subjects. The addition of sodium phytate to white bread—in amounts comparable with those found in brown bread—inhibited the absorption of calcium fully as much as did the brown bread. Calciferol (2,000 units per diem) made very little difference to the calcium metabolism on brown bread dietaries. The addition of suitable amounts of calcium carbonate (Creta preparata) to the brown bread raised the amount of calcium absorbed. The fortification could not be detected by appearance or taste, and has been recommended.

As the above two communications concerned different aspects of the same investigation they were discussed together. PROFESSOR R. A. PETERS thought that the taste of bread was improved by the addition of calcium. COLONEL H. L. TIDY felt that intestinal flatulence was a considerable disadvantage of these diets, and he was far from convinced that the ordinary person would take to them as enthusiastically as the clinical scientist. DR. R. E. SMITH thought that further experiments were necessary on adolescents before approving of such diets.

### Luncheon

Luncheon was held in Magdalen College, and the President proposed the health of the Association, congratulating members on the success of the meeting and welcoming the guests and visitors.

### 3 p.m. Afternoon Session

1. SIR ARTHUR HURST and DR. A. H. T. ROBB-SMITH (introduced) showed the intestines of a woman of 52 years who had died of uncontrollable diarrhoea. She was found to have a living tape-worm in her duodenum and jejunum, which were severely inflamed and showed eosinophilic infiltration. It was then learnt from her husband that she had been said to have a tape-worm 25 years before. No record of a similar case could be found.

SIR ARTHUR HURST also spoke on *Pitressin in Intestinal Flatulence*. He described the treatment of chronic gaseous distension of the colon in the absence of starch dyspepsia by the daily injection of pitressin, and showed radiographs to demonstrate the action of the drug.

2. PROFESSOR L. J. WITTS made *Some Observations on Purpura Haemorrhagica*, saying that the clotting time of the plasma was slightly prolonged when measured by Howell's test. Tissue extracts promoting coagulation consisted of water-soluble and fat-soluble moieties, and if their action were analysed by the use of solutions of Russell viper venom and lecithin, it appeared that there was a deficiency in the fat-soluble factor in thrombocytopenia.

COLONEL H. L. TIDY said that the coagulation time in purpura haemorrhagica was not prolonged unless the platelets were reduced.

3. LT.-COLONEL W. S. C. COPEMAN described a case which appeared to be one of *Pseudo-Spondylitis Ankylopoietica* in which no abnormality could be found to account for the patient's posture, and in which it was assumed that the lesion was due to contractures resulting from nitrogen emboli affecting the lumbar aponeurosis whilst working in caissons at high pressure. There was no evidence of any hysterical factor. He gave a summary of the present position of knowledge with regard to the 'rheumatic' sequelae of working in compressed air. In this group he discussed acute myalgia ('bends'), arthralgia, bursitis, and the arthritic sequelae of nitrogen emboli of the nutrient arteries of the epiphyses of long bones. He concluded that workers in pressures of less than 2.4 atmospheres absolute did not suffer, but that in all the higher pressures the damage could be minimized if the workers could be made to realize the importance of prolonging the period of decompression.

SIR ARTHUR HURST said that this was a typical example of the 'bent back of soldiers', and DR. GORDON HOLMES agreed that it was a case of hysterical contracture which should be curable in 5 or 10 minutes. COLONEL H. L. TIDY was of the same opinion.

4. PROFESSOR H. J. SEDDON (introduced by SIR FARQUHAR BUZZARD) spoke on *Nerve Regeneration in Man*. He described work in progress on rates of regeneration of peripheral nerves after injuries of various types. The most convenient pathological classification was the following, (i) complete division of nerve fibres, every part of the nerve trunk being severed, (ii) a 'lesion in continuity'—division of axons, but preservation of more or less of the supporting structures—a type of lesion easily produced experimentally by heavy crushing of a nerve. Most injuries to nerves could be identified sooner or later as belonging to one or other type, or a mixture of the two. In experimental animals Mr. J. Z. Young had found that the rate of regeneration after crushing was more rapid than after complete division, and evidence was produced which suggested that the same was true in man. A third type described as 'transient block' was characterized by a loss of conduction due to a lesion which did not lead to Wallerian degeneration. The only form of injury known to produce this kind of lesion was compression (e.g. crutch palsy). Recovery is always rapid. It is possible that the morbid changes are similar to those found in diphtheritic neuritis.

DR. F. J. NATTRASS pointed out that it was important, but very difficult, to differentiate between the first and second types of lesion.

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